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**Towards an integrated pest
management in quinoa in traditional
and new production zones of Peru**

By

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Thesis submitted in fulfillment of the requirements for the
degree of Doctor (PhD) in Bioscience Engineering: Agriculture

Dutch translation of the title:

Naar een geïntegreerde plaagbeheersing in quinoa in traditionele en nieuwe productiezones van Peru

Please refer to this work as follows:

Cruces, L. 2022. Towards an integrated pest management in quinoa in traditional and new production zones of Peru. PhD thesis, Ghent University, Ghent, Belgium.

ISBN-number: 978-94-6357-519-5

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This work is dedicated to three women who have been my source of personal and professional motivation: My mother Carmen Navarro, and my professors Clorinda Vergara and Luz Gómez.

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List of abbreviations

ABC:	Augmentative Biological Control
ACE:	abundance-based coverage estimator
ANOVA:	analysis of variance
CBC:	Conservation Biological Control
DD:	degree-days
DNA:	deoxyribonucleic acid
df:	degrees of freedom
EIL:	economic injury level
ETL:	economic threshold level
F:	the statistic used in an ANOVA
FAO:	Food and Agriculture Organization of the United Nations
IGR:	insect growth regulator
IPM:	Integrated Pest Management
IRAC:	Insecticide Resistance Action Committee
IYQ:	International Year of Quinoa
K:	degree-day requirements
L:D:	light-dark cycle expressed in hours
m a.s.l.	meters above sea level
MANOVA:	Multivariate analysis of variance
NMDS:	non-metric multidimensional scaling
p:	p-value, significance of a statistical test
PCR:	polymerase chain reaction
RH:	relative humidity
R ² :	coefficient of determination
SE:	standard error
SENAMHI	National Service of Meteorology and Hydrology (a Peruvian institution)
SENASA	National Service of Agrarian Sanitation (a Peruvian institution)
SIMPER:	similarity percentage, a type of analysis that calculates the contribution of each species (%) to the dissimilarity between groups

t_0 : lower development threshold (°C)
 χ^2 : Chi-square test.
W: Mann-Whitney statistic value

Chapter 1

General introduction, objectives and thesis outline

1.1. General introduction

Human consumption patterns are changing at a global scale, and the demand for healthier options of food is increasing, particularly in developed countries. Consequently, new markets are opened to cover the increasing demand of these nutritional products (Bazile et al., 2016; Alandia et al., 2020).

Quinoa (*Chenopodium quinoa* Willd.) is one of those nutritional products that is getting an increasing interest by the international market. This pseudocereal, rich in lysine, vitamins, calcium, magnesium, copper, phosphorous, potassium and zinc, and containing no gluten (See Annex 1), has also drawn attention in developing countries, where nutritional problems in weaning babies and children are endemic issues (Repo-Carrasco et al., 2003; Abugoch, 2009; Gómez & Aguilar, 2016).

For a long time, quinoa was a neglected and underutilized species, only connected to the Andes where it was also considered by the local population as a marginal crop, not only due to the unawareness of its nutritional properties but also because its consumption was associated to the poorest segments of the society (Mujica, 1994; Bazile et al., 2016; Bedoya-Perales et al., 2018a). However, as a result of the increasing attention of the international scientific community, the multiple benefits of this crop have been unravelled (e.g. tolerance to drought, salinity and cold; high nutritional value; adaptation to different environments) (Bazile et al., 2016; Gómez & Aguilar, 2016; Bedoya-Perales et al., 2018a).

The increasing demand for quinoa has led to an extraordinary expansion of the cultivated area in Peru, the so-called “quinoa boom”, which started in 2009 but became noteworthy in 2013 and 2014. Thus, its domestic consumption passed from being a little valued crop to achieving prominence (Bedoya-Perales et al., 2018a, 2018b; Gamboa et al., 2018; McDonell, 2019); and Peru became the main producer of quinoa worldwide in 2020 with 100,115 tons, followed by Bolivia with 70,170 tons and Ecuador with 4,903 tons (FAO, 2022).

However, the quinoa expansion also had negative implications (Walsh-Dilley, 2016). One of the consequences was the crop displacement, i.e. when quinoa became a more profitable crop, the cultivation in the highlands of other native staple foods such as canhigua (*Chenopodium pallidicaule* Aellen), tarwi (*Lupinus mutabilis* Sweet), oca (*Oxalis tuberosa* Molina) and mashua (*Tropaeolum tuberosum* Ruiz & Pavón), began to significantly decrease. These crops (among others) were usually included in a crop rotation system or in a mixed cropping, but during the quinoa boom the land-use shifted to quinoa monocultures, reducing the benefits of traditional cropping systems and their genetic diversity in the Andean valleys. Moreover, in the Peruvian

coastal region, quinoa emerged as a new crop, displacing during 2013 to 2015 other typical crops from the zone, but such displacement did not occur with the same magnitude as it happened in the highlands (Gómez-Pando et al., 2014; Bedoya-Perales et al., 2018a; Gómez-Pando et al., 2019; SIEA, 2022).

In the Peruvian coastal region, improved agricultural techniques were used in quinoa cultivation, e.g. agricultural machinery, technified irrigation, chemical fertilizers and phytosanitary products were intensively applied, raising the efficiency of production, and resulting in yields twice as high as those obtained in the traditional producing regions in the Andes (Gómez-Pando et al., 2014; Bedoya-Perales et al., 2018a; Gómez-Pando et al., 2019). However, soon after the beginning of cultivation of quinoa in the lowlands, farmers had to deal with another negative implication of the quinoa expansion, the emergence of pests which were not previously known to infest quinoa (including the heteropterans *Liorhyssus hyalinus* (Fabricius) and *Nysius simulans* Stål) (Cruces et al., 2016; Latorre, 2017). This phytosanitary issue arose because of the unplanned and disorderly expansion of the crop, which occurred in different regions of Peru; for example, during the cropping season 2014 in the district of Majes, Arequipa province, farmers were sowing quinoa at any time during the year, to the point that in the same valley there were quinoa fields of different phenological stages (from newly sown fields to fields about to be harvested) where pests could find food and refuge everywhere (Castle, 1999; Soto et al., 2015; Latorre, 2017).

The disorderly quinoa expansion led to the adoption of harmful control measures, such as the excessive use of pesticides. Without knowing the biological parameters of the relatively new pests, most of such measures were applied “blindly”, affecting the natural enemies of pests which in turn may cause pest resurgence and/or the emergence of new pests (Wilby & Thomas, 2002; Bedoya-Perales et al., 2018a). The smallholder farmers from the Peruvian coastal areas usually rely on agrochemicals to keep pest populations at minimal levels. However, sometimes they overuse pesticides, in terms of applied doses and frequency, without taking into account recommended economic threshold levels and without caring about the alternation of chemicals with different modes of action. This has led to resistance to certain agrochemicals. Furthermore, such chemical compounds can contaminate the soil, affecting fertility due to the declining populations of beneficial organisms in the soil and disrupting the ecological services provided by the beneficial organisms (Mahmood et al., 2016; Latorre, 2017; Bedoya-Perales et al., 2018a; Ramirez-Hernandez et al., 2020).

Given the situation outlined, a need has arisen to apply better farming practices in order to prevent damage by the pests with minimal risks to the environment and human health

(Mohammad, 2014; Rapisarda & Cocuzza, 2017). Exploring better alternatives of chemical and biological control in the frame of an Integrated Pest Management (IPM) program may support long-term sustainability of agricultural production of quinoa in the lowlands (Singh & Sharma, 2004). This implies a suitable combination of pest control methods to reduce economic damage on crops by setting action thresholds, correctly identifying and monitoring pests, as well as preventing and suppressing pests in a cost-effective manner and with the least possible environmental hazard (Mohammad, 2014; Reddy, 2014).

Under an IPM scheme, biodiversity plays an important role (Altieri & Nicholls, 2004; Frison et al., 2011; Mohammad, 2014). Even in agroecosystems where only one species of plant (the crop) prevails, high diversity, particularly of insects, can be expected. Insect diversity in crops is formed by different feeding guilds (including herbivorous and carnivorous species) that are part of a complex food chain (Altieri, 1999; Ramani, 2013). In the first step of the present study, the insect diversity associated with quinoa in the lowlands as compared to the highlands of Peru is explored by using pitfall traps. This is one of the most popular techniques used to study the arthropods (Leather, 2005; Saska et al., 2013), which has proved to be efficient to analyse the diversity in both natural vegetation and agroecosystems (Gill & Mc Sorley, 2012; Callohuari et al., 2018; De la Cruz et al., 2019; Livia & Sánchez, 2019; Livia & Sánchez 2020; Livia et al., 2020; Socca, 2020). This contributes to identifying (in part) the local biodiversity that needs to be protected or conserved, and to set a basis to measure impacts of agricultural intensification onwards. Besides, plant samplings were done to analyse the incidence of the major pests and their natural enemies, which contribute to providing baseline data for an integrated pest management in quinoa.

Among the pest control methods applied, probably the use of the insecticides has the highest negative impact on insect diversity, reducing mainly the diversity of the natural enemies (Forister et al., 2019). Moreover, the more widely used insecticides by smallholder farmers in Peru belong to the pyrethroids, organophosphates and carbamates groups, all of them of broad spectrum compounds (Wagner et al., 2016; Cruz, 2017; Latorre, 2017; Delgado-Zegarra et al., 2018). Since the correct use of selective insecticides is an essential component of Conservation Biological Control (CBC) (Ramani, 2013), the current study analyses the effects of two broad spectrum insecticides as compared to two selective insecticides on the prevalence of quinoa pests. Also, their side effects on the arthropod diversity and on the occurrence of the key natural enemies were studied by combining three widely used sampling methodologies: pitfall traps, pan traps and plant sampling (Leather, 2005).

As in other invertebrates, temperature is one of the environmental variables with the highest influence on insect performance, affecting the physiology of immature and adult insects and therefore also their population dynamics (Chown & Nicolson, 2004; Price et al., 2011; Schowalter, 2016). For these reasons, the thermal biology of two poorly known newly emerged quinoa pests, *L. hyalinus* and *N. simulans*, is addressed in the current study. The data obtained may allow to infer potential occurrences of these species in different production areas, thus being essential tools when developing IPM programs.

Chemical control remains an important tool in IPM. However, when the application of insecticides involves risks of harvesting products with residues, biological control may take a crucial role in the management of pests (van Lenteren, 2000; Giles et al., 2017; Baker et al., 2020). For example, the heteropteran pests *L. hyalinus* and *N. simulans* infest quinoa at the grain filling stage, when the application of insecticides may contaminate the grains. This results in pesticide residues in the harvested quinoa, which may eventually lead to rejection of the harvest product by an importing country due to the potential health risks for its consumers and international regulation on pesticide residues (Cruces et al., 2016). In the current study, *Chrysoperla externa*, a common lacewing that occurs in quinoa and other crops in the lowlands of Peru (Sánchez & Vergara, 2005; Valoy et al., 2015) was chosen to evaluate its potential as a biological control agent of these true bugs.

1.2. Objective and thesis outline

The overall objective of this thesis research was to explore the diversity of the harmful and beneficial entomofauna of quinoa cultivated in traditional and new production zones of Peru and to explore alternatives of pest control under an IPM scheme.

Firstly, field observations were performed in three altitudinal production zones, including one traditional (from the Andes) and two non-traditional quinoa production areas at lower elevations, one at the “Maritime Yunga” ecoregion and other at the coastal level. Next, a field trial at the coastal level was performed in order to determine the impact of insecticides on quinoa pests and their side effects on the natural enemies. This was followed by laboratory experiments to study the developmental and reproductive biology of two major pests, *L. hyalinus* and *N. simulans* as well as to assess the predation potential of the lacewing *C. externa* as a potential biological control agent of these heteropterans. These objectives can be translated into the following research questions:

- How different is the insect diversity at the coastal level as compared to the quinoa production zones at higher elevations?
- What is the incidence of the major pests of quinoa at the coastal level as compared to that cultivated in the Andean region?
- What is the impact of two broad spectrum insecticides (imidacloprid and cypermethrin) widely use by Peruvian farmers at the coastal level, as compared to two selective insecticides (teflubenzuron and emamectin benzoate), against the main quinoa pests and what are their side effect on non-target species?
- What is the effect of the temperature on development and reproduction of the hemipteran quinoa pests *L. hyalinus* and *N. simulans*?
- What are the predation capacity of *C. externa* larvae and their developmental parameters on *L. hyalinus* and *N. simulans*? What is the preference of *C. externa* larvae when offered the aphid *Macrosiphum euphorbiae* (Thomas), *L. hyalinus* or *N. simulans*?

The research questions are addressed in several chapters: **Chapter 2** provides a background on the ecozones where quinoa is cultivated, on the characteristics of the crop, its cultivation, varieties, and on the major pests of quinoa in the lowlands. **Chapter 3** highlights the differences in the entomofauna composition and insect diversity between quinoa cultivated at the coastal level and quinoa cultivated at higher elevations (i.e. in the “Maritime Yunga” ecozone at 1,410 m a.s.l. and in the Andean region at 3,322 m a.s.l.), while **Chapter 4** describes the occurrence of quinoa pests and their natural enemies in these three altitudinal production zones. In **Chapter 5** the impact of four insecticides with different selectivity and modes of action against major quinoa pests and their side effects on non-target species are studied. In **Chapter 6** the development and reproduction of *L. hyalinus* and *N. simulans* are studied at several constant temperatures and thermal requirements are estimated. **Chapter 7** focuses on the predation capacity and development of *C. externa* larvae on *L. hyalinus* and *N. simulans*, and studies the prey preference among *M. euphorbiae*, *L. hyalinus* and *N. simulans*. The final section, **Chapter 8**, gives a general discussion of the findings and provides further research perspectives.

Chapter 2

Quinoa and its major pests in the lowlands of Peru: A literature review

2.1. The Andean region

The Andes comprises an extensive mountain chain that runs from the north to the south of South America, through the countries of Venezuela, Colombia, Ecuador, Peru, Bolivia, Chile, and Argentina (Figure 2.1). It stretches over an area 8,500 km long and 250-750 km wide; the average altitude is between 3,000 and 4,000 m a.s.l. with the highest peak up to near 7,000 m a.s.l. (Guerrero et al., 2011; Borsdorf & Stadel, 2015; Cohen-Aponte, 2019).



Figure 2.1. Map of South America displaying the Andean region (map: Mapswire, CC BY 4.0).

2.2. Quinoa: an ancestral crop in the Andean region

Quinoa is a crop cultivated since ancient times in the Andean region, mainly in Bolivia and Peru and to a lesser extent in Argentina, Chile and Ecuador. This pseudo-cereal was one of the main foods for the Andean settlers in the pre-Hispanic period until the South-American conquest time, when the introduction of cereals such as wheat, barley and oats surrogated quinoa. Around 500 years after the Spanish conquest, the cultivated area of quinoa suffered a dramatic reduction, particularly in the inter-Andean valleys of the central and northern highlands (2,300 – 3,500 m a.s.l.). However, its production at higher elevations (over 3,500 m a.s.l.) in the Altiplano zone, has been maintained along the years to present; as a result, this agroecological zone has the highest genetic diversity of quinoa (Eiselen, 1956; Tapia, 1979; Maughan et al., 2007; Gandarillas et al., 2014; Gómez-Pando et al., 2014; Gómez & Aguilar, 2016).

2.3. Global expansion of quinoa and its implications

For many years, and until a couple of decades ago, quinoa has been considered as a marginal crop, used in self-subsistence farming, for farmer family consumption or for feeding their animals (i.e. cattle, pork, sheep, chicken). It has mainly been cultivated at a small scale, in plots of usually less than 2 ha. This situation still remains in a large part of the highlands (Eiselen, 1956; Tapia, 1979; Gandarillas et al., 2014).

Over the last two decades, quinoa has been gaining international interest for food security and nutrition due to its resilience and high nutritional value (Jacobsen et al., 2003; Abugoch, 2009; FAO & Bioversity international, 2012; Basantes-Morales et al., 2019). By the end of the 1980s, this crop was produced in 11 countries outside of the Andes. By the end of 2012, 30 more countries cultivated the Andean grain for research and production. In 2013, the Food and Agricultural Organization of the United Nations (FAO) declared the International Year of Quinoa (IYQ), and it was during this year that great efforts were done to promote quinoa consumption (Bazile & Baudron, 2014; Bazile et al., 2016; Cruces et al., 2016; Alandia et al., 2020).

With higher quinoa demands, its national and international market value increased and export prices rose to more than fivefold, making quinoa a profitable crop. This motivated more farmers in South America to grow this grain, even far away from its Andean origins at lower elevations, leading to a considerable increase of the cultivation area. In Peru, the quinoa harvested area increased from 28,889 ha (2000) to 67,638 ha (2020); in Bolivia, it increased from 36,847 ha (2000) to 115,973 ha (2020); whereas in Ecuador, the increase was from 1,300 ha (2000) to 5,267 ha (2020) (Cruces et al., 2016; FAO, 2022). The quinoa expansion also occurred outside of the Andean region: by 2018, the crop was cultivated for research and production in 123 countries (Alandia et al., 2020).

In the Andes of Peru, many farmers motivated by higher revenues switched staple crops such as native tubers and grains, legumes, or corn, to quinoa. At lower elevations, where quinoa was an unusual crop, this grain started to be grown at a large scale (Gómez-Pando et al., 2014; Cruces et al., 2016; Gómez & Aguilar, 2016). In this context, in 2014 there was an overproduction of quinoa which made the market prices collapse; in that year, 200 tons of quinoa were rejected by the United States because pesticide residues were found to be above the maximum threshold, causing a negative impact on the reputation of Peruvian quinoa. Moreover, a new pest complex emerged in these non-traditional quinoa production zones, where it became a less profitable crop. In this context and with new competitors (non-Andean countries), by the end of 2015 smallholders had to face prices as low as ten times less than in 2013 (Alandia et al., 2020).

The abovementioned situation unmotivated Peruvian farmers from the “Coast” and “Maritime Yunga” regions to continue growing quinoa, resulting in a significant gradual reduction of the planted area of the crop in these zones. Thus, the surface of quinoa on the Coast was reduced from 6,137 ha in 2014, to 770 ha in 2020, whereas in the “Maritime Yunga”, it fell from 7,487 ha to 620 ha (SIEA, 2022).

Nowadays, although prices do not reach high peaks as before, quinoa can be considered as a profitable crop. Moreover, increasing food trends such as veganism, vegetarianism and flexitarianism have created market windows where organic quinoa, which has a higher price in the market as compared with conventional quinoa, is considered an important source of high quality plant-based proteins and can also be sold on Fairtrade terms (Alandia et al., 2020; <https://www.fairtrade.net>).

2.4. Taxonomy of quinoa

Quinoa has the following classification (Catalogue of life, 2022):

KINGDOM: Plantae
PHYLUM: Tracheophyta
CLASS: Magnoliopsida
ORDER: Caryophyllales
FAMILY: Amaranthaceae
SUBFAMILY: Chenopodioideae
GENUS: *Chenopodium*
SPECIES: *quinoa* Willd.

Quinoa belongs to the subfamily Chenopodioideae, previously treated as a separated family from Amaranthaceae, but continued studies on their morphology and phylogeny revealed their closeness which resulting in their merging (Mroczek, 2015). The family Amaranthaceae comprises about 160 genera and 2,400 species, which includes crops such as *Beta vulgaris* L., *Spinacea oleracea* L., and *Amaranthus caudatus* L. as well as ornamental plants belonging to the genera *Salsola*, *Celosia*, *Iresine* among others (Labronici-Bertin et al., 2013; Eshete et al., 2016; Bayoumy et al., 2020). This family also includes numerous species of weeds, among them *Portulaca oleracea* L., *Chenopodium murale* (L.), *Chenopodium album* L., *Amaranthus dubius* Mart. and *Amaranthus spinosus* L. (Gómez & Aguilar, 2016).

2.5. Agroecology of quinoa

2.5.1. Phenology

The quinoa life cycle is 6 months on average, but this period may vary depending on the cultivar and region where quinoa is cultivated. In general terms, the grain is harvested from 83 (early varieties) to 190 (late varieties) days after sowing (Gómez & Aguilar, 2016; Sosa-Zuniga et al., 2017).

According to the scale of the Biologische Bundesanstalt Bundessortenamt und Chemische Industrie (BBCH), quinoa has 10 principal growth stages, which are summarized in Table 2.1 (Mujica & Canahua, 1989; Gómez & Aguilar, 2016; Sosa-Zuniga et al., 2017). The main growth stages are displayed in Figure 2.2.

Table 2.1. Growth stages of quinoa based on the BBCH scale and their main characteristics.

Code	Growth stage	Main characteristics
0	Germination	The cotyledons emerge from the soil, still joined.
1	Leaf development	Opposite paired leaves are extended in a rhomboid way.
2	Formation of side shoots (secondary stems)	Opposite paired shoots become visible (pair by pair). Branching begins.
3	Stem elongation	
4	Development of harvestable vegetative parts	
5	Inflorescence emergence	Inflorescence appears, protected by the leaves at the beginning. Inflorescence emerges surrounded by small leaves. The basal leaves become progressively yellowish.
6	Flowering	Beginning of flowering to full bloom. Flowering anthesis.
7	Fruit development	Ovary thickening and first visible grains in the main stem.
8	Ripening/maturity fruit	Milky grain: liquid content and green pericarp. This phase is critical because the plant is sensitive to hydric deficit. Thick grain: white pasty content, green, beige, red or black pericarp. Ripe grain: hard grain, dry content, the grain has a beige, red or black colour and it is ready to harvest.
9	Senescence	The moisture of the grain is about 15%. The whole plant becomes yellowish and there is an intense defoliation.

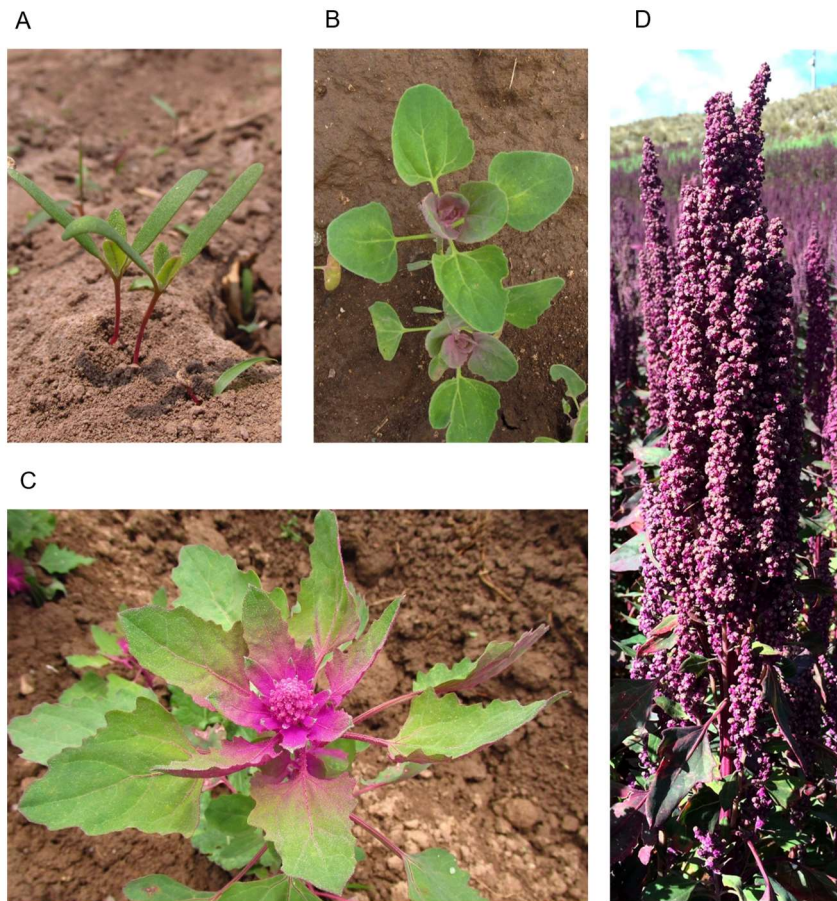


Figure 2.2. Some main growth stages of quinoa: A. Germination; B. Leaf development; C. Inflorescence emergence; D. Ripening (Photos: author).

2.5.2. Agroecological zones

In the Andean countries, quinoa is cultivated in five agroecological zones along the altitudinal gradient where varieties with specific characteristics have developed. These zones are Altiplano, inter-Andean valleys, Yunga, Salares and Sea level (Gómez-Pando et al., 2014; Rojas & Patiño, 2014; Gómez & Aguilar, 2016).

2.5.2.1. Altiplano

At an altitude over 3,500 m a.s.l., this zone is located in the hydrographic basin of Lake Titicaca, belonging to Peru and Bolivia (see Annex 2). In this agroecological zone, there are usually large variations between day and night temperatures, the annual mean temperature is around 7.3 °C and the annual mean precipitation is around 500 to 600 mm. Adverse environmental conditions such as drought, frost or hail may occur during the coldest months. In Peru, this zone covers the major area of quinoa production (>54% in 2020), located in the department of Puno (See Annex 3) (Gómez-Pando et al., 2014; Rojas & Patiño, 2014; SIEA, 2022).

Most of the traditional and commercial varieties originate from this zone, where the major quinoa diversity has developed. Quinoa plants here usually have simple stems, without branches, the panicle is compact, grains are saponin-rich, and the plants are highly susceptible to mildew (*Peronospora variabilis* Gäum) (Gómez & Aguilar, 2016).

2.5.2.2. Inter-Andean Valleys

This zone is located at an altitude between 2,300 and 3,500 m a.s.l. (see Annex 2). The weather is very variable, depending on the altitude and season, although periods of drought and high precipitation throughout the cropping season are frequent (annual mean precipitation is around 500 mm). Adverse environmental conditions such as frost and hail may also occur, which during the ripening stage reduce the quinoa yields. In Peru, these valleys are located in the departments of Cusco and Apurímac (in the southern part), Junín and Ayacucho (in the central part), and Ancash and Cajamarca (in the northern part) (see Annex 3) (Gómez-Pando et al., 2014; Rojas & Patiño, 2014).

Quinoa plants that evolved in this agroecological zone are usually branched, with a loose to semi-compact panicle, grains are saponin-low, and some varieties may express a certain degree of resistance/tolerance to mildew (*P. variabilis*) (Gómez & Aguilar, 2016).

2.5.2.3. Yunga

In Bolivia, at an altitude between 1,500 and 2,200 m a.s.l., a small group of quinoas have adapted to the environmental conditions of the mountainous jungle. However, quinoa production in this zone is of minor importance (Rojas & Patiño, 2014).

In Peru, over the last years, the quinoa crop has been grown in the agroecological zone called “Yunga Maritima” (see Annex 2), which corresponds to the transitional zone of the highlands to the lowlands, between 500 and 2,300 m a.s.l. The climate in this zone is moderately warm, slightly humid with scarce precipitation. The main irrigation projects in this zone are “San Camilo”, “Santa Rita de Sigwas” and “Majes”, in the department of Arequipa, where diverse commercial varieties are extensively cultivated (See Annex 3) (Gómez-Pando et al., 2014).

2.5.2.4. Salares

This zone is located in the salt flats of the southern Altiplano of Bolivia and the northern Altiplano of Chile, at 4,000 m a.s.l. The zone is desertic, with particular agroecological conditions such as a soil pH above 8.0 and low annual precipitation (200-300 mm), which are conditions in which no other crops can thrive (Gómez-Pando et al., 2014; Rojas & Patiño, 2014).

Quinoa plants here are similar to the Altiplano type. In this zone varieties with the largest grains have developed, and they are known as “royal quinoa” or “quinoa real”. The predominating varieties have grains with a thick pericarp and rich in saponins (Gómez & Aguilar, 2016).

2.5.2.5. Sea level

In the southern and central coastal areas of Chile, where soils are poor and degraded, quinoa is cultivated at a small scale mainly for self-consumption. These quinoa plants are branched, with small, yellow translucent grains and high saponin content (Rojas & Patiño, 2014; Gómez & Aguilar, 2016).

In Peru, in recent years, the coastal region has become a new production zone (see Annex 2). Departments where quinoa has been grown during the last decade are Tacna, Moquegua, Ica, Lima, La Libertad, Lambayeque and Piura. Commercial varieties with diverse characteristics have been cultivated in this agroecological zone (See Annex 3) (Gómez-Pando et al., 2014; Gómez & Aguilar, 2016).

2.6. Quinoa cropping systems

2.6.1. Traditional system

Quinoa is cultivated under the traditional system in the highlands (the Altiplano or inter-Andean valleys agroecological zones) where mostly organic production is done. In this system, there is an intense use of labour from soil preparation (sometimes aided by animal traction) to the harvest (Figure 2.3 A, B). This cultivation is mostly rainfed irrigated, although there are accessible zones, near the rivers, where complementary surface irrigation can be applied (Gómez-Pando et al., 2014).

Traditional quinoa cultivation usually involves a crop rotation system or crop associations with corn, beans, broad beans, cucurbits, potatoes, tarwi and other native grains and tubers (Figure 2.3 A). A common agricultural practice is to prepare the soil just after the harvest, in order to take advantage of the humidity that remains from the raining season; after preparation, farmers leave the field in such conditions for 2 to 4 months until the beginning of the next cropping season. Another regular practice is organic fertilization, with the incorporation of manure from the cattle in the fields (Gómez-Pando et al., 2014; Basantes-Morales et al., 2019; Alandia et al., 2020).



Figure 2.3. Farmers from the department of Huancavelica (in the highlands of Peru) cultivating quinoa under two cultivation systems; A. crop association, with legumes; B. monoculture (Photos: author).

2.6.2. Modern system

Modern systems involve monocultures which can be grown under organic (mostly in the highlands) or conventional production systems (mostly in new production areas).

Recently, more advanced farming techniques have been applied in quinoa production in zones outside of the highlands (i.e., Yunga Maritima and Sea level ecoregions). Soil preparation is done with agricultural machinery, irrigation can be surface or technified and the use of chemical inputs (fertilizers and pesticides) is high and systematic, which sometimes includes modern equipment (Figure 2.4) (Gómez-Pando et al., 2014; Gómez & Aguilar, 2016; Cruces et al., 2016; Latorre, 2017).



Figure 2.4. Quinoa cultivation in non-traditional systems: A. Cultivation and hilling; B. Drip irrigation; C. Chemical control with spray equipment; D. Threshing (Photos: author).

2.7. Commercial varieties of quinoa

Table 2.2 provides a list of commercial Peruvian varieties with a short general description and the recommended zone of cultivation (Gómez & Aguilar, 2016; Basantes-Morales et al., 2019).

Figure 2.5 shows some commercial varieties in the field.

Table 2.2. Commercial Peruvian varieties with main characteristics of the grain and region where they can be cultivated.

Name	Flavor base on Saponin content*	Grain color		Size	Recommended agroecological zone
		Pericarp	Episperm		
INIA 431-Altiplano	Sweet	Creamy	White	Large	Altiplano, Cost
INIA 427-Amarilla sacaca	Bitter	Yellow	White	Large	inter Andean valleys
INIA 420-Negra ccollana	Sweet	Grey	Black	Small	Altiplano, inter Andean valleys, Cost
INIA 415-Pasankalla	Sweet	Grey	Red	Medium	Altiplano, inter Andean valleys, Cost
Illpa INIA	Sweet	Creamy	White	Large	Altiplano
INIA Salcedo	Sweet	Creamy	White	Large	Altiplano, inter Andean valleys, Cost
Qillahuaman INIA	Semi-Sweet	Creamy	White	Medium	inter Andean valleys
Ayacuchana INIA	Semi-Sweet	Creamy	White	Small	inter Andean valleys
Amarilla de Marangani	Bitter	Orange	White	Large	inter Andean valleys
Blanca de Juli	Semi-Sweet	Creamy	White	Small	Altiplano
Blanca de Junín	Semi-Sweet	Creamy	White	Medium	inter Andean valleys, Cost
Cheweca	Semi-Sweet	Creamy	White	Medium	Altiplano
Huacariz	Semi-Sweet	Creamy	White	Medium	inter Andean valleys
Blanca de Hualhuas	Sweet	Creamy	White	Medium	inter Andean valleys
Huancayo	Semi-Sweet	Creamy	Creamy	Medium	inter Andean valleys
Kankolla	Semi-Sweet	Creamy	White	Medium	Altiplano
Mantaro	Sweet	Creamy	Creamy	Medium	inter Andean valleys
Rosada de Junín	Semi-Sweet	Creamy	White	Small	inter Andean valleys
Rosada de Taraco	Bitter	Creamy	White	Small	Altiplano
Rosada de Yanamango	Semi-Sweet	Creamy	White	Medium	inter Andean valleys
La Molina 89	Semi-Sweet	Creamy	White	Medium	inter Andean valleys, Coast

* High saponin content gives bitter flavour.



Figure 2.5. Peruvian varieties of quinoa: A. INIA 415-Pasankalla; B. Amarilla de Marangani; C. Blanca de Hualhuas; D. Rosada de Huancayo; E. La Molina 89; F. INIA Salcedo (Photos: author).

2.8. Major pests of quinoa in the lowlands of Peru

Around 78 arthropods have been recorded to infest quinoa in South America, but most of these phytophagous species are of minor importance. In the highlands of Peru, *Eurysacca melanocampta* (Meyrick) and *Eurysacca quinoa* Povolný (Lepidoptera: Gelechiidae) are considered the key pests. Other pests that can be major importance in the Andean regions are *Helicoverpa quinoa* Pogue & Harp and *Copitarsia 19 ncommode* (Walker) (Lepidoptera: Noctuidae) (Povolný, 1997; Rasmussen et al., 2001; Dughetti et al., 2013; Saravia et al., 2014; Dughetti, 2015°; Cruces et al., 2016).

Quinoa crops in the lowlands of Peru can be severely infested by a wider range of phytophagous insects, but none of them have been reported as key pests yet. This pest complex includes *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae), *Liorhyssus hyalinus* (Fabricius) (Hemiptera: Rhopalidae), *Nysius simulans* Stål (Hemiptera: Lygaeidae), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae), *E. melanocampta* and *Spoladea recurvalis* (Fabricius) (Lepidoptera: Crambidae). Occasionally, *Chloridea virescens* (Fabricius) and *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae) may infest the quinoa plants in high densities (Cruces et al., 2016; Cruces et al, 2020a).

Economic impact in terms of yield reduction has been only studied for *E. melanocampta*, while for the other pests there is still a knowledge gap to be filled.

2.8.1. *Macrosiphum euphorbiae* (Thomas)

2.8.1.1. Taxonomy

This species, commonly known as the potato aphid, has the following classification (Quirós et al., 2009):

CLASS :	Insecta
ORDER :	Hemiptera
SUB-ORDER:	Sternorrhyncha
FAMILY:	Aphididae
GENUS :	<i>Macrosiphum</i>
SPECIES:	<i>euphorbiae</i> (Thomas, 1878)

2.8.1.2. Distribution

Macrosiphum euphorbiae is native to North America and it has a cosmopolitan distribution (Blackman & Eastop, 2017). In the Neotropical Region the aphid is found in Argentina, Brazil, Bolivia, Caribbean islands, Chile, Colombia, Ecuador, Peru and Venezuela (Heie et al., 1996).

2.8.1.3. Morphology

Nymphs are very similar to the apterous adults, but with a less shiny cuticle due to the wax that covers the body. The general colour is yellowish green or yellowish pink; the cornicles are long, with dark tips (van Emden, 2013; Capinera, 2020).

Adults vary in appearance, in forms (wingless or winged) and colour (pink or green). The most common is the apterous parthenogenetic form, whose body is green or pink, without markings; legs and cornicles have the same colour. The cornicles are quite long, terete, dark at the apex; the cauda is also quite long; the antennal tubercles diverge or point outward (Figure 2.6). Body length of the apterous adults is 3.0 – 4.0 mm. The adult winged parthenogenetic form has the same pink or green body with cornicles darker at the tips, but bears transparent wings with dusky veins; body length is 2.1 – 3.4 mm (Wilca, 2010; Blackman & Eastop, 2017; Capinera, 2020). Identification keys are given by Blackman & Eastop (2000, 2006).



Figure 2.6. *M. euphorbiae* adult. A. Dorsal view; B. Lateral view (Photos: Y. Callohuari).

2.8.1.4. Life cycle

Under laboratory conditions at 22 °C, 70 ± 10% RH, and a 12 h photoperiod, the total nymphal development on lettuce (*Lactuca sativa* L.) averages 7.3 days, while at 16 °C, it takes 10.8 days. Temperatures above 28 °C have detrimental effects on the aphids, and a mortality of 100% was observed at 31 °C (De Conti et al., 2011).

2.8.1.5. Host plants

At least 200 species of hosts have been reported, mainly of the families Amaranthaceae, Apiaceae, Asteraceae, Brassicaceae, Convolvulaceae, Cucurbitaceae, Fabaceae, Liliaceae, Malvaceae, Poaceae, Solanaceae and Rosaceae (Blackman & Eastop, 2006; Van Emden, 2013). *M. euphorbiae* infests quinoa, lettuce, tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), rose (*Rosa* spp.), sweetpotato (*Ipomoea batatas* L.), cotton (*Gossypium* spp.), alfalfa (*Medicago sativa* L.), peach (*Prunus persica* (L.) Stokes), hibiscus (*Hibiscus rosa-sinensis* L.), artichoke (*Cynara scolymus* L.), bean (*Phaseolus vulgaris* L.) and peas (*Pisum sativum* L.) (Sánchez & Vergara, 2003; Vilca & Vergara, 2011).

2.8.1.6. Behaviour and crop damage

Colonies of *M. euphorbiae* infest the underside of the leaves, buds, flower primordia and inflorescences of quinoa (Figure 2.7 A). Nymphs and adults suck phloem sap, weakening the plants and causing wilting. As a consequence of their honeydew production, fungal proliferation of sooty mold may cover the leaves and panicle (Figure 2.7 B, C) (Sánchez & Vergara, 2002).

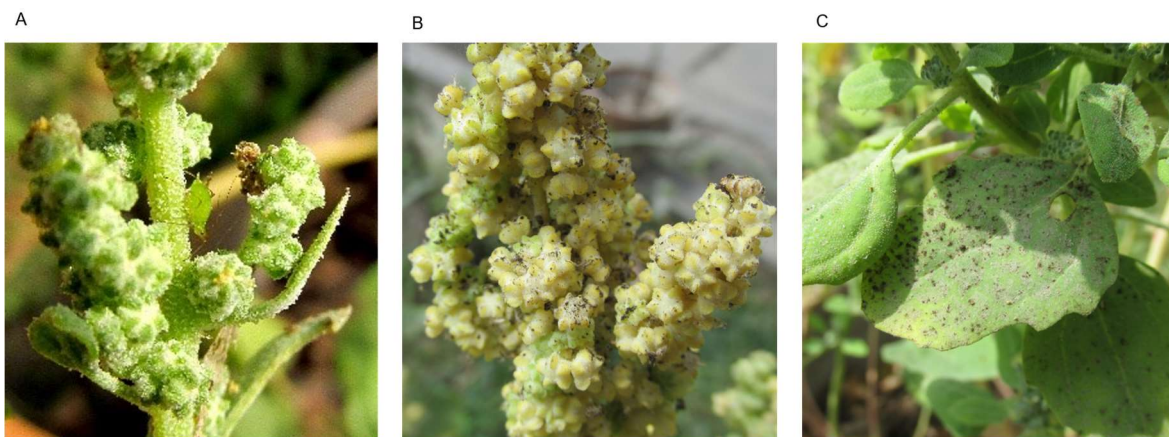


Figure 2.7. *M. euphorbiae*: A. Adult female on panicle; B. Panicle with sooty mould; C. Leaves with sooty mould (Photos: author).

2.8.2. *Liorhyssus hyalinus* (Fabricius)

2.8.2.1. Taxonomy

This species, known as the scentless plant bug or quinoa true bug, has the following classification (Cornelis et al., 2012; Fowles et al., 2015):

CLASS :	Insecta
ORDER :	Hemiptera
SUB-ORDER:	Heteroptera
FAMILY:	Rhopalidae
GENUS :	<i>Liorhyssus</i>
SPECIES:	<i>hyalinus</i> (Fabricius, 1974)

2.8.2.2. Distribution

This is a cosmopolitan species (Göllner-Scheiding, 1976). In South America, it is reported from Argentina, Chile, Ecuador, Venezuela and Peru (Alata-Cóndor, 1973; Froeschner, 1981; Cermeli et al., 2004; Prado, 2008; Dughetti, 2015a).

2.8.2.3. Morphology

Eggs are red, ovoid, slightly depressed laterally. Length of the eggs average 0.85 mm (Figure 2.8 A). They are deposited in clusters of 10 to 40 eggs (Vilímová & Rohanová, 2010; Cornelis et al., 2012).

First instar nymphs have a reddish brown head, thorax and legs and brilliant red abdomen; their body surface covered with dark brown hairs (Figure 2.8 B). Second instars are brownish red, with darker antennae and legs; their body surface is covered with whitish pilosity and bears sparse long brown hairs. The wing pads appear in the third instar and increase in size up to the fifth instar. Older instar nymphs are light brown to ochre, bearing abundant whitish pilosity over the surface and sparse long brown hairs. The length of mature nymphs averages 4.62 mm (Figure 2.8 C) (Cornelis et al., 2012).

The adult is ovoid, without a dense pubescence. The general coloration is usually ochre, but different morphotypes can be seen in the field (with individuals varying from reddish to dark brown). The head bears a longitudinal medial yellow line and has some spots between the eyes; the antennae are ochre. The pronotum has a longitudinal medial yellow line, connected to the medial line of the head and extending toward the scutellum. The membrane of the hemelytron

is hyaline, exceeding the apex of the abdomen. The legs are yellow, bearing numerous dark spots. The body length ranges from 5.5 to 6.5 mm (Figure 2.8 D) (Steill & Meyer, 2003).

An identification key is provided by Göllner-Scheiding (1976).

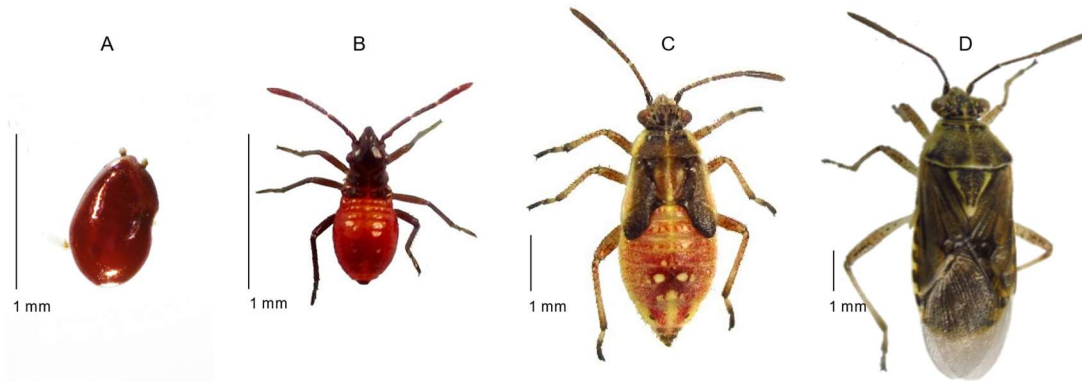


Figure 2.8. Life stages of *L. hyalinus*: A. Eggs; B. First instar nymph; C. Fifth instar nymph; D. Adult female (Photos: L. Cruces; Y. Callohuari).

2.8.2.4. Life cycle

At 25 °C, the egg incubation period averages 5 days, the nymphal development takes 14 to 18 days and adults can live longer than two months. At this temperature, the female lays up to 193 eggs, although higher fecundity has been reported at 34 °C (278 eggs) (Hradil et al., 2007).

2.8.2.5. Host plants

This is a polyphagous species, but in Peru it has been reported as an important pest only in quinoa. However, members of the family Amaranthaceae, Asteraceae, Cucurbitaceae, Fabaceae, Euphorbiaceae, Malvaceae, and Poaceae can also be hosts (Wheeler, 2016).

2.8.2.6. Behaviour and crop damage

The adults are good flyers. They have a diurnal activity and can mate several times across their lives. Eggs are laid in clusters on the leaves, on developing quinoa grains, and less commonly on the stems (Figure 2.9 A, B) (Hradil et al., 2007; Cruces et al., 2016).

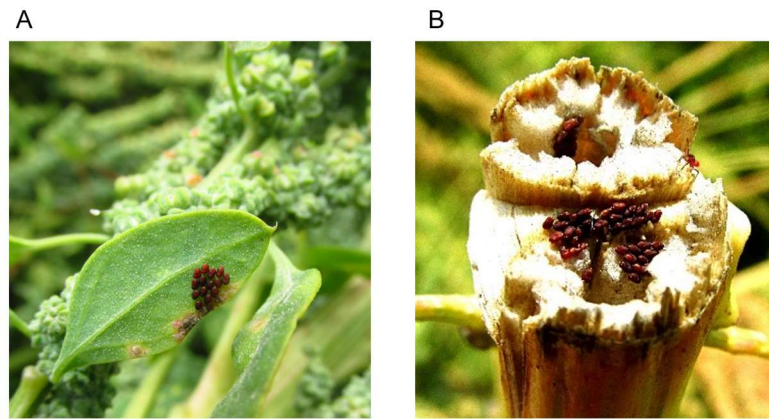


Figure 2.9. *L. hyalinus* oviposition: A. On the leaf; B. On a broken stem (Photos: author).

First nymphal instars start sucking the plants just after hatching. They have a gregarious behaviour until the fifth instar, and they usually are observed in the panicle, between the developing grains. *L. hyalinus* may feed on the different plant parts, but usually the highest infestation occurs during the grain formation when the nymphs and adults attack the grains, causing direct damage by reducing the grain weight (Figure 2.10 A-C) (Hradil et al., 2007; Dughetti, 2015a; Cruces et al., 2016).



Figure 2.10. *L. hyalinus* infesting the panicle of quinoa (A-C) (Photos: Y. Callohuari; L. Gómez; L. Cruces).

2.8.3. *Nysius simulans* Stål

2.8.3.1. Taxonomy

This species, known as the false chinch bug or minute quinoa bug, has the following classification (Henry et al., 2015):

CLASS :	Insecta
ORDER :	Hemiptera
SUB-ORDER:	Heteroptera
FAMILY:	Lygaeidae
GENUS :	<i>Nysius</i>
SPECIES:	<i>simulans</i> Stål 1859

2.8.3.2. Distribution

This is a neotropical species, distributed in Argentina, Brazil, Chile, Peru, Paraguay and Uruguay (Dalazen et al., 2014; Cruces et al., 2020b; Chorbadjian et al., 2021).

2.8.3.3. Morphology

Eggs are elongated, rice-like in shape (tapered towards the ends), yellowish and creamy. They are laid singly or in small groups. Length of the eggs averages 0.91 mm (Figure 2.11 A) (Cruces et al., 2016; Maquera, 2018).

First instar nymphs have a brown head bearing yellow and brown longitudinal stripes, in subsequent instars, the longitudinal stripes become more notorious; their antennae are brown with paler intersegmental joints; the pronotum is dark brown, bearing a middle pale-yellow stripe; the eyes are prominent. The abdomen is pale yellow with the intersegmental joints being orange to red in colour (Figure 2.11 B). The wing pads become visible from the third instar and increase in size as the nymph grows (Dughetti, 2015b).

Adults have a greyish brown dorsal surface of head, pronotum and scutellum. The antennae and legs are yellow and bear dark spots. The eyes are prominent and dark reddish, the two ocelli are reddish. The corium and clavus of the forewing are light ochre with dark brown spots, the membrane is translucent and marked with rows and small spots. Body length averages 4 mm (Figure 2.11 C) (Dughetti, 2015b; Pall et al., 2016).

An identification key is given by Pall et al. (2016).

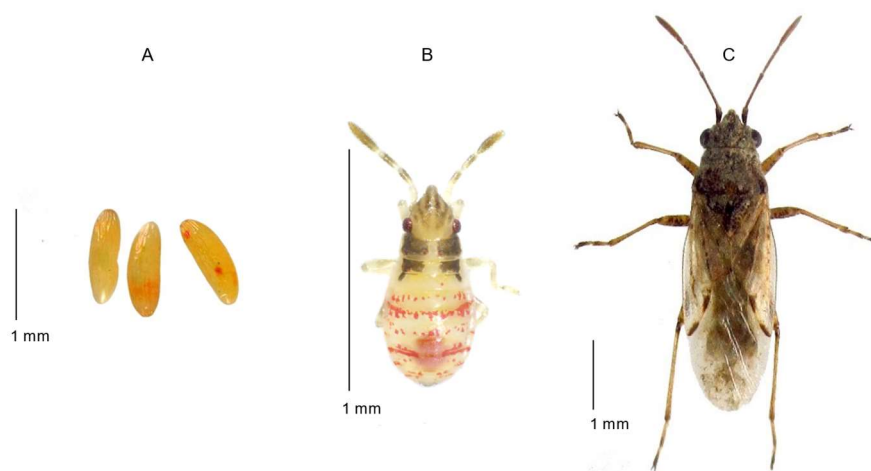


Figure 2.11. Life stages of *N. simulans*: A. Eggs; B. First instar nymph; C. Adult (Photos: L. Cruces, Y. Callohuari)

2.8.3.4. Host plants

This is a polyphagous pest, feeding mainly on Amaranthaceae, Asteraceae, Brassicaceae, Fabaceae, Malvaceae, Poaceae and Solanaceae. In Peru, it has been reported as a pest in quinoa, asparagus (*Asparagus officinalis* L.) and kiwicha (*Amaranthus caudatus* L.), whereas in other countries of South America crops such as lettuce, potato, rice (*Oryza sativa* L.), corn (*Zea mays* L.), wheat (*Triticum* spp.), cotton (*Gossypium* spp.), soybean (*Glycine max* L.) and strawberry (*Fragaria × ananassa* (Weston) Rozier) have also been reported as hosts (Costa-Lima, 1940; Alata-Cóndor, 1973, Di Iorio, 2004; Aragón & Flores, 2006; Montero *et al.*, 2007, Dalazen *et al.*, 2014; Dughetti, 2015a, 2015b).

2.8.3.5. Behaviour and crop damage

They are soil-surface dwelling insects and show diurnal activity. Before flowering of quinoa, nymphs and adults remain on the ground and on low-growing weeds. During the grain filling stage, they climb into the quinoa plants to feed on the developing grains, disrupting the seed formation and thus directly affecting the yield (Figure 2.12, 2.13) (Dughetti, 2015b).

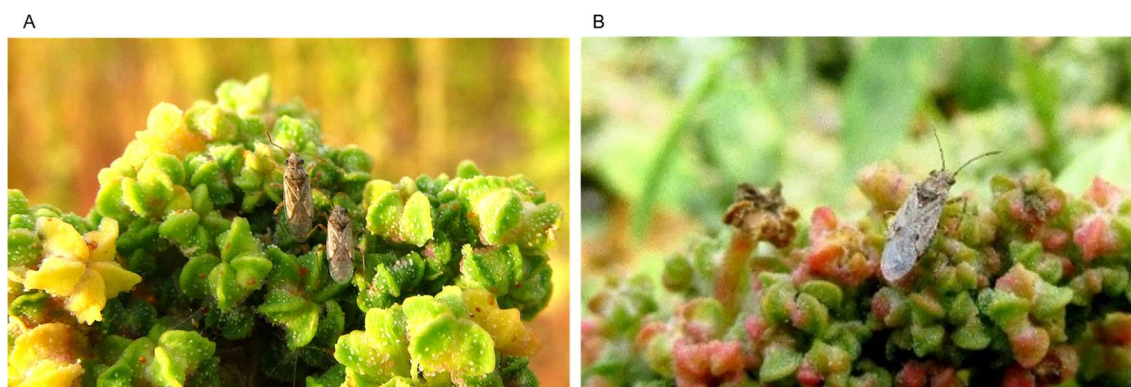


Figure 2.12. *N. simulans* on panicle of quinoa (Photos: author).

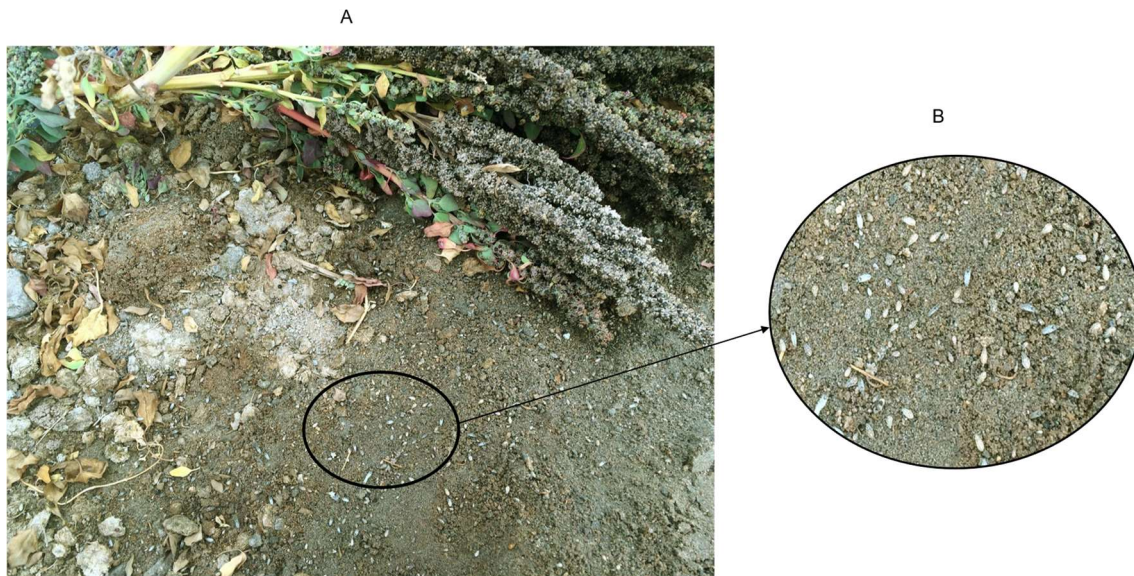


Figure 2.13. *N. simulans* in a quinoa field. A. Adults and nymphs gathering on the ground; B. Enlarged section (Photo: author).

2.8.4. *Frankliniella occidentalis* (Pergande)

2.8.4.1. Taxonomy

The western flower thrips has the following classification (Parker et al., 1995; Mound & Ng, 2009):

CLASS :	Insecta
ORDER :	Thysanoptera
SUBORDER:	Terebrantia
FAMILY:	Thripidae
GENUS :	<i>Frankliniella</i>
SPECIES:	<i>occidentalis</i> (Pergande, 1895)

2.8.4.2. Distribution

F. occidentalis is a cosmopolitan species, extensively expanded around the world, distributed in at least 57 countries (Monteiro et al., 1999; He et al., 2020). It best survives in warm climates but has a relatively high tolerance to cold climates (Capinera, 2020).

2.8.4.3. Morphology

This hemimetabolous species has the following developmental pattern: Egg, larva I, larva II, prepupa, pupa and adult:

Eggs are inserted into the plant tissue by an ovipositor and can be found on leaves, flowers or fruits. They are white, bean-like, about 0.20 - 0.25 mm long (Childers & Achor, 1995; Capinera, 2020).

Larva I and Larva II are creamy white to yellowish, spindle-like, without wing pads. Setal patterns on the abdomen can be used to differentiate the first and second instar, as well as the sexes (Nakahara, 1993).

Prepupae and pupae are usually white to cream coloured. Prepupae have distinguished short wing pads and erect antennae. Pupae have long wing pads that reach almost to the tip of the abdomen, and their antennae are bent backward along the head (Capinera, 2020).

Adults vary in colour, from yellow to brown: females are usually larger and darker than the males (yellowish white) (Figure 2.14). Their body is slender, the antennae bear eight segments and the wings are fringed, along the anterior edge of the wings fringes are markedly shorter than on the posterior edge. The pronotum has typical long setae. Body length of adults range from 1.2 to 1.9 mm (Capinera, 2020; CABI, 2021).

An identification key is given by Mound & Kibby (1998).

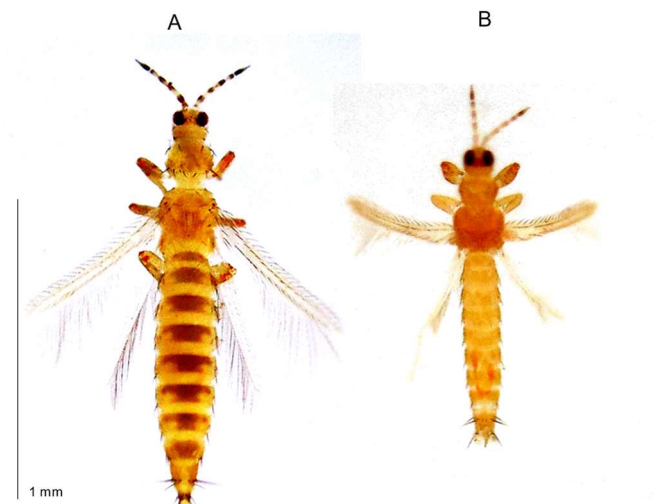


Figure 2.14. *F. occidentalis*. A. Female; B. Male (Photos: Y. Callohuari).

2.8.4.4. Life cycle

At 25 °C, the egg incubation period averages 2.6 days, first and second larval instars require about 2.3 and 3.7 days, respectively; the prepupal and pupal development take 1.1 and 2.7 days, respectively. Female fecundity is up to 190 eggs (Trichilo & Leigh, 1988; Capinera, 2020). Adults can live from 20 to 70 days, depending on temperature (Capinera, 2020).

2.8.4.5. Host plants

This species has a wide range of host plants, and it is considered an important pest worldwide in tomato, lettuce, bean, peach, apple (*Malus domestica* Borkh.), avocado (*Persea americana* Mill.), squash (*Cucurbita maxima* Duchesne), cucumber (*Cucurbita pepo* L.), onion (*Allium cepa* L.), pepper (*Capsicum* spp.), blueberry (*Vaccinium corymbosum* L.) among several others crops, weeds and wild plants (Capinera, 2020).

2.8.4.6. Behaviour and crop damage

Females can reproduce parthenogenetically (producing only males) or by mating (producing both sexes) (Capinera, 2020).

Larval stages I and II are the feeding stages, actively feeding on young vegetative and reproductive parts; they usually are found in concealed places on the plants, such as within flowers or developing leaves, or under the calyx of fruits. When larval development is completed, they drop to the ground to pupate. Prepupa and pupa are nonfeeding stages and they may be found on the soil surface, in cracks and crevices; although capable of movement, neither pupal stage moves about actively unless disturbed (Hansen et al., 2003; Capinera, 2020).

F. occidentalis is considered a major pest on a wide range of crops where they cause damage by causing flowers to shed, by malforming fruits, and by mottling and distorting leaves which reduces the production of photosynthates. This damage is a consequence of the feeding behaviour, which consist of scraping the epidermis with their left mandibula stylet, and then piercing and sucking the sap with their maxillae (Heming, 1978; Capinera, 2020). Probably the major injury of this species is as a vector of plant viruses, which may cause severe diseases in crops (e.g. tomato-spotted wilt virus) (Mound & Marullo, 1996; Reitz, 2009).

In quinoa, these thrips infest the crop from the flowering stage to ripening. Although high densities can be found on the quinoa plants, economic injury in terms of yield reduction has not been reported (Cranshaw et al., 1990; Saravia et al., 2014; Chapter 4).

2.8.5. *Liriomyza huidobrensis* (Blanchard)

2.8.5.1. Taxonomy

This species, commonly known as the serpentine leafminer fly, has the following classification (Korytkowski, 2014):

CLASS :	Insecta
ORDER :	Diptera
FAMILY :	Agromyzidae
GENUS :	<i>Liriomyza</i>
SPECIES :	<i>huidobrensis</i> (Blanchard, 1926)

There was a misconception in the literature regarding the identity of *Liriomyza langei* and *L. huidobrensis* due to their morphological similarities, and therefore, they were considered as synonyms by Spencer (1973). Later, Scheffer & Lewis (2001), using molecular techniques, identified these species as different taxa.

2.8.5.2. Distribution

This species is native to the neotropics, but currently it is distributed worldwide, in countries of America, Africa, Asia and Europe (Echevarria et al., 1994; Scheffer, 2000; Scheffer & Lewis, 2001; López et al., 2010; Capinera, 2020).

2.8.5.3. Morphology

The eggs are white, oval in shape, 0.28 mm long and 0.15 mm wide. They are endophytic, inserted into the leaf tissue (Capinera, 2020).

The larvae are colourless after hatching, turning to pale yellow to orangish yellow as they mature. They are acephalous and apodous, the posterior spiracles each have a regular ellipse of 6 to 9 tiny pores. The length of mature larvae averages 3.3 mm (Spencer, 1973; Sánchez & Vergara, 2003; Capinera, 2020).

Newly puparia are yellowish, turning light reddish brown to blackish in colour as they are closer to the emergence of the adult. The length of the pupa is 1.6-3.2 mm (Spencer, 1973; Capinera, 2020).

The adults are dark brown to black. Head with frons, vertex and gena, yellow in colour. The thorax has a shining black mesonotum and its hind angles bear a yellow patch that joins the scutellum; the upper edge of the mesopleuron is yellow, the inferior part is black; the scutellum

is yellow with brown to black basal corners; the femora are yellow, with irregular brown spots; tibia and tarsus are dark brown. Abdominal terga and sternae are blackish brown, and the intersegmental membrane is yellow; abdominal pleura are yellow. Body length is 1.7-2.1 mm and wingspan 1.9 to 2.5 mm (Spencer, 1973; Korytkowski, 2014; Capinera, 2020).

Identification keys are given by Spencer (1973) and Korytkowski (2014).

2.8.5.4. Life cycle

The most optimal temperature for development, survival and reproduction is between 20 and 25 °C; hotter temperatures are more detrimental than colder temperatures (Lanzoni et al., 2002; Mujica et al., 2017). At laboratory conditions (20 °C, 60 % RH, 12 h photoperiod), egg incubation period averages 3.4 days; larval and pupal development take 6.4 and 11.9 days on average, respectively; adult longevity is 8.3 – 8.9 days. The female fecundity is 94.7 eggs, which is significantly affected by high (around 30 °C or more) and low temperatures (around 15 °C or less) (Salas et al., 1988; Hincapie et al., 1993; Mujica et al., 2017).

2.8.5.5. Host plants

This is a polyphagous pest that infests at least 14 plant families. In Peru, this leaf miner fly is an important pest in quinoa, tomato, potato, peas, artichoke, lettuce, among others belonging to the families Alliaceae, Amaranthaceae, Apiaceae, Asteraceae, Caryophyllaceae, Cucurbitaceae, Fabaceae, Malvaceae and Solanaceae (Spencer, 1990; Wei et al., 2000; Sánchez & Vergara, 2003; Korytkowski, 2014).

2.8.5.6. Behaviour and crop damage

The adults have a diurnal activity, flying actively during sunny hours. Copulation occurs between 6 and 24 hours after the emergence of the adults. Oviposition takes place generally in the mornings and the eggs are inserted into the epidermal tissue of the leaves (Figure 2.15 A, B) (Parrella et al., 1981; Sánchez & Vergara, 2003). Females use their pseudo-ovipositor to lacerate the plant tissue, after which both females and males feed on the emanating sap (Capinera, 2020).

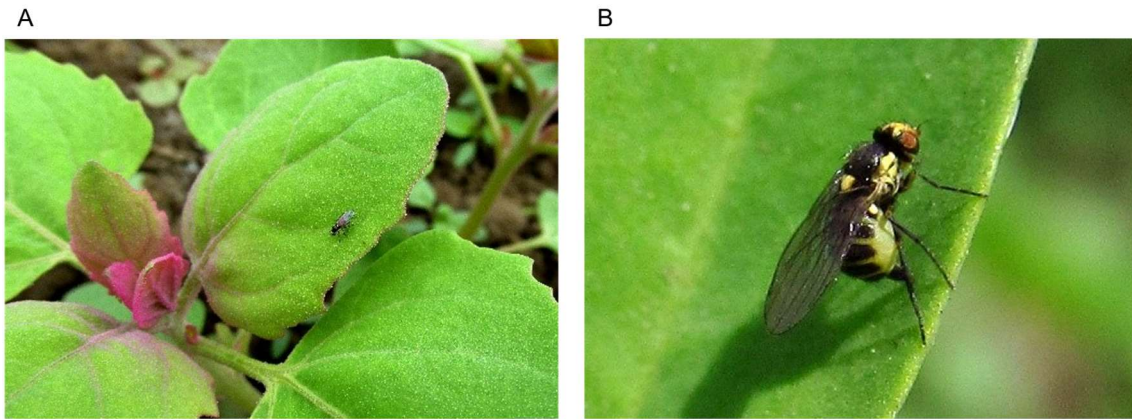


Figure 2.15. *L. huidobrensis*: A. Adult on quinoa leaf; B. Adult female inserting its pseudo-ovipositor into the leaf tissue (Photos: L. Cruces; Y. Callohuari).

When the infestation occurs at germination, females puncture on the cotyledonal leaves (Figure 2.16 A). When infestation occurs at the later stages of the crop phenology, the punctures are observed in the upper part of the plants (Figure 2.16 B) while larval damage occurs in the lower parts.

Larvae feed in the spongy mesophyll tissue and produce long twisting mines that widen as the larvae mature. High incidence of larvae in the leaves may cause an intense decolouration and senescence, with a subsequent defoliation. When larval development is completed, larvae leave the mines and drop to the ground to form a puparium in the soil (Wilcox & Howland, 1955; Spencer, 1973; Sánchez & Vergara, 2003; Cruces et al., 2016; Capinera, 2020).

As a consequence of larval feeding, the photosynthetic capacity of the leaves is reduced (Figure 2.16 C) (Sánchez & Vergara, 2003).

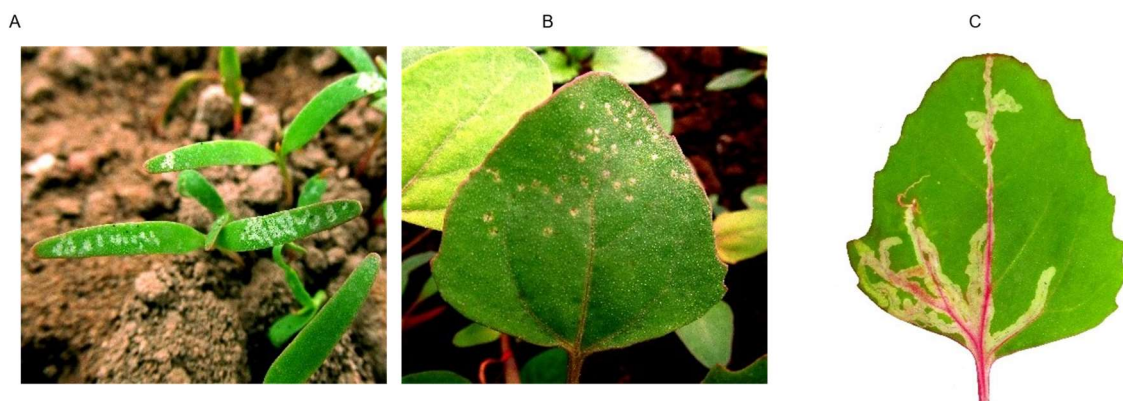


Figure. 2.16. *L. huidobrensis* damage. A. Adult damage on cotyledonal leaves; B. Adult damage on a true leaf; C. Larval damage on a true leaf (Photos: L. Cruces; Y. Callohuari).

2.8.6. *Eurysacca melanocampta* (Meyrick)

2.8.6.1. Taxonomy

The quinoa moth, has the following classification (Povolný, 1986):

CLASS :	Insecta
ORDER :	Lepidoptera
FAMILY :	Gelechiidae
GENUS :	<i>Eurysacca</i>
SPECIES:	<i>melanocampta</i> (Meyrick, 1917)

2.8.6.2. Distribution

E. melanocampta is present in Argentina, Bolivia, Chile, Colombia, Ecuador, and Peru (Povolný & Valencia, 1986; Povolný, 1990, 1997; Lamborot et al., 1999; Rasmussen et al., 2003; Valoy et al., 2011).

2.8.6.3. Morphology

Eggs are pale yellow, 0.22 mm long, oval in shape, and smooth. They are deposited singly or in small groups. (Figure 2.17 A) (Carrera et al., 2016).

First instar larvae are yellowish, with diffuse maculae. Later instar larvae are greenish yellow to dark brown, bearing reddish spots throughout the body. The head is pale yellow to dark brown. Length of the mature larvae averages 10.2 mm (Figure 2.17 B) (Carrera et al., 2016).

Pupae are reddish brown; the apex of the proboscis reaches the abdominal segment VI. Cremaster is triangular, bearing a hook-like process at the tip. Length averages 5.6-5.7 mm (Figure 2.17 C) (Carrera et al., 2016).

Adults are grey; forewings brownish grey, bearing a longitudinal narrow dark band near the coastal margin and two oval spots in the centre of the wing. The head bears abundant scales on the frons and vertex; antennae are filiform; labial palpi are broad, curve forward and upward; maxillary palpi are very small. Body length ranges from 6 to 9 mm and wingspan is 12.3 - 12.5 mm (Figure 2.17 D) (Carrera et al., 2016).

A detailed morphological description is given by Povolný (1986).

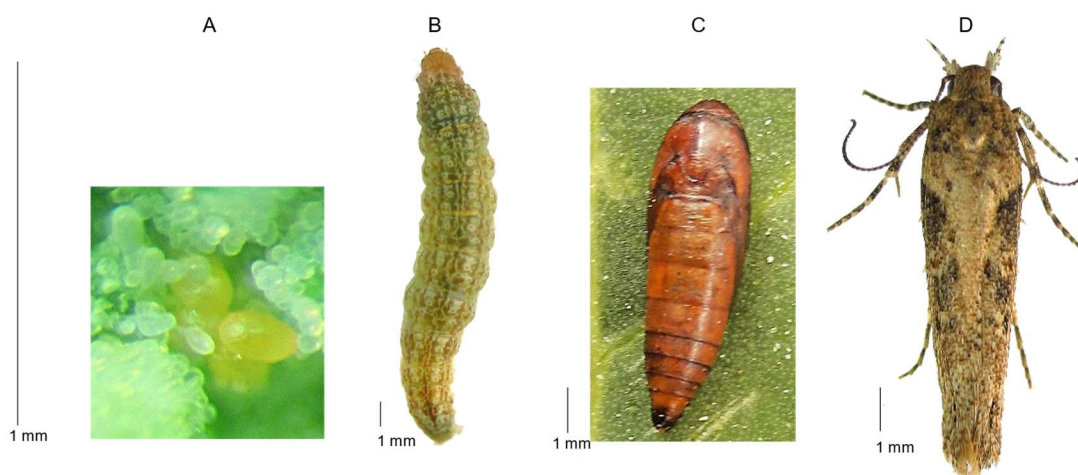


Figure 2.17. Life stages of *E. melanocampta*: A. Egg; B. Larva; C. Pupa; D. Adult (Photos: L. Cruces; Y. Callohuari).

2.8.6.4. Life cycle

Under laboratory conditions (20 ± 3 °C, $60 \pm 5\%$ RH, 12 h photoperiod), the egg incubation period averages 7 days, larvae and pupae complete their development in 27 and 20 days, respectively, and adult longevity is about 21 days. Female fecundity is around 300 eggs (Quispe et al., 2014).

2.8.6.5. Host plants

This species is an important pest only in quinoa, but kiwicha, kanihua (*Chenopodium pallidicaule* Aellen), broad bean (*Vicia faba* L.) and tarwi (*Lupinus mutabilis* Sweet) have been reported as hosts (Chacón-Galindo, 1963; Povolý, 1979; Carrasco, 1987; Quispe et al., 2014).

2.8.6.6. Behaviour and crop damage

Adults have a crepuscular and nocturnal activity; during the day, they remain hidden and quiet on the leaves, inflorescence or panicle (Ochoa-Vizarreta & Franco-Navia, 2013) (Figure 2.18). The females deposit their eggs on the underside of the leaves, or near or on the inflorescence in case of infestations at flowering stage (Sánchez & Vergara, 2002).

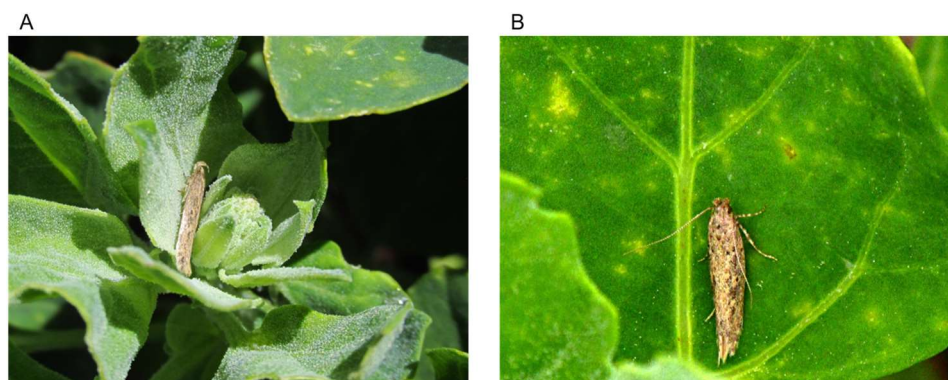


Figure 2.18. *E. melanocampta*: A. and B. adult on quinoa leaves (Photos: author).

When *E. melanocampta* infests quinoa plants at early stages, the larvae spin the apical leaves together, which serves as a shelter, and inside they feed on the apical buds (Figure 2.19 A); first instars may also feed in the mesophyll tissue and produce blotch mines on the foliage (Figure 2.19 B). As the panicle grows during the flowering stage, the larvae leave the leaf and enter inside the panicle where they feed on the developing grains (Figure 2.19 C, D) (Quispe et al., 2014). When larval development is completed, some caterpillars go down into the soil to pupate while others complete their pupal development inside the panicle (Sánchez & Vergara, 2002).

Indirect damage of *E. melanocampta* on the production of quinoa grains is caused when the larvae feed on the leaves, reducing the photosynthetic capacity of the plants. Direct damage occurs when larvae feed on the grains, causing up to 60% of yield loss when the pest density reaches 30 larvae per plant (Quispe et al., 2014).

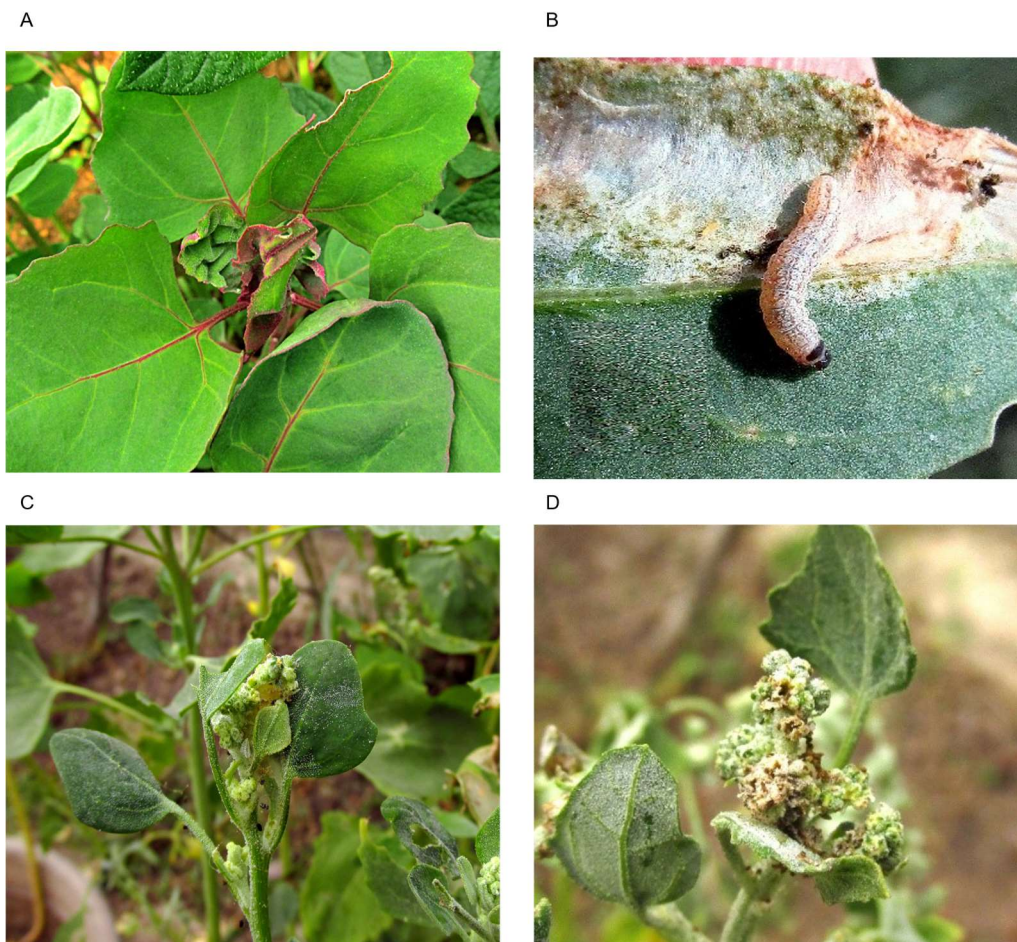


Figure 2.19. *E. melanocampta* damage: A. Apical leaves spun together; B. Blotch mine on a leaf opened to expose the larva; C Leaves stuck to part of the inflorescence; D. Part of the panicle with feeding damage (Photos: author).

2.8.7. *Spoladea recurvalis* (Fabricius)

2.8.7.1. Taxonomy

This species, known as the Hawaiian beet webworm, has the following classification (Scholtens & Solis, 2015; Capinera, 2020):

CLASS :	Insecta
ORDER :	Lepidoptera
FAMILY:	Crambidae
GENUS :	<i>Spoladea</i>
SPECIES:	<i>recurvalis</i> (Fabricius, 1775)

2.8.7.2. Distribution

S. recurvalis occurs in Europa, Asia, Africa, Oceania (Australia and New Zealand), America (but without records in Argentina, Bolivia and Ecuador). The highest incidence is in tropical and subtropical regions (Vasantharaj & Ananthakrishnan, 2004; Muthaiyan, 2009; Powell & Opler, 2009; CABI, 2020).

2.8.7.3. Morphology

Eggs are bluish, scale-like (flattened), deposited singly or in small clusters on the underside of the leaves, adjacent to large veins. Eggs are 0.6 mm long, 0.5 mm wide and 0.25 mm thick (McDougall et al., 2013; Capinera, 2020).

First instar larvae are pale green, difficult to spot in the foliage. As larvae reach maturity, the green colouration becomes more intense, with a visible medial dark green band along the dorsum, accompanied with white stripes laterally. The mesonotum has two dark spots, behind the prothoracic shield. The head is ochre to brown, with lateral and dorsal areas bearing small golden spots (Figure 2.20 A, Figure 2.21 A, B). When larvae are close to pupation, they become uniformly pinkish or rust coloured. Length of the mature larvae averages 18.4 mm (Brier, 2007; McDougall et al., 2013; Capinera, 2020).

Pupae are oblong, light brown. The posterior end bears terminal spines with hooked tips. Length of the pupae is 9.0 - 9.5 mm (Pande, 1969; Capinera, 2020).

Adults are dark brown with a purple tint. The fore wings bear two transversal white bands and two small circular white spots near the external margin; the hind wings have only one oblique

white band which runs along the central area (Figure 2.20 B). The wingspan is 17-23 mm (Brier, 2007; McDougall et al., 2013; Capinera, 2020).

An identification key is given by Solis (2006).



Figure 2.20. Life stages of *S. recurvalis*. A. Larva; B. Adult (Photos: L. Cruces; Y. Callohuari).

2.8.7.4. Life cycle

Under laboratory conditions at 25 ± 2 °C, egg incubation averages 3.7 days; the larval period (when fed on *Trianthema monogyna* and *Amaranthus viridis*) takes 14.4 days and the pupal period 8.4 days (Pande, 1972). Fecundity of females is up to 200 eggs (Miller, 2019).

2.8.7.5. Host plants

In Peru, *S. recurvalis* usually infests quinoa, Kanihua, beetroot (*Beta vulgaris* L.), and spinach (*Spinacia oleracea* L.), whereas a wide range of weeds and wild plants of the family Amaranthaceae, Apiaceae, Asteraceae, Brassicaceae, Fabaceae, Juglandaceae, Poaceae and Rutaceae can also be hosts (Solis, 2006; Brier, 2007; Powell & Opler, 2009; Capinera, 2020).

2.8.7.6. Behaviour and crop damage

Adults are crepuscular. During the day they remain hidden in the shade in the lower parts of the plants. Eggs are deposited singly or in batches on the undersides of leaves. Emerging larvae feed on the lower surface of the foliage, not eating entirely through the leaves; more mature larvae consume the entire leaf and infest the panicle to also feed on the developing grains (Figure 2.21 A, B). They spin the leaves together or spin a leaf to the panicle to shelter and feed inside. At high densities, it is common to see abundant feces and silk produced by the larvae on the leaves and branches (Figure 2.21 C) (Sánchez & Vergara, 2002; Capinera, 2020).

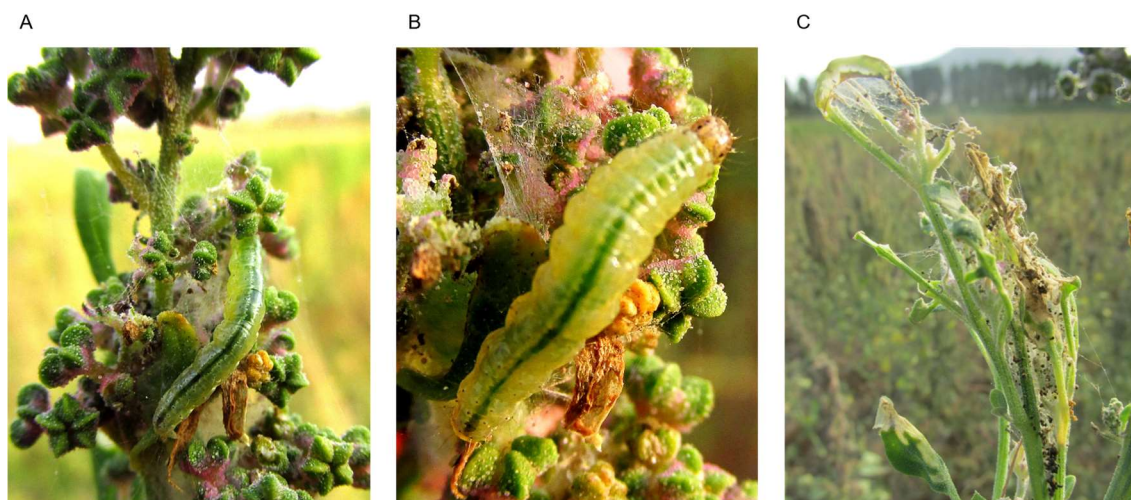


Figure 2.21. Damage by *S. recurvalis* on quinoa (Photos: author).

2.8.8. *Chloridea virescens* (Fabricius)

2.8.8.1. Taxonomy

This species, commonly known as the tobacco budworm, has the following classification (Pogue, 2013; Capinera, 2020):

CLASS :	Insecta
ORDER :	Lepidoptera
FAMILY :	Noctuidae
GENUS :	<i>Chloridea</i>
SPECIES:	<i>virescens</i> (Fabricius, 1777)

This species was widely known in the literature as *Heliothis virescens*, but was placed by Pogue (2013) within the genus *Chloridea*.

2.8.8.2. Distribution

In America, this species is distributed in the United States, Mexico, Nicaragua, El Salvador, Argentina, Brazil, Colombia, Paraguay, Peru, Uruguay, Venezuela, Cuba, Jamaica and the Caribbean. In Europe it has been reported from England (Muthaiyan, 2009; Capinera, 2020).

2.8.8.3. Morphology

Eggs are yellowish white, turning to grey as they mature. They are sub-conical, with radial striae and are deposited singly (Figure 2.22 A). The diameter of the egg at its base is 0.51-0.60 mm and the length is 0.5-0.61 mm (Angulo et al., 2006; Carrera, 2013; Capinera, 2020).

Larvae are polychromatic, usually of green tints, but there are also whitish, pinkish, ochre, reddish or blackish forms, bearing always three longitudinal darker bands dorsally; the lateral bands are usually more pronounced than the central band, which mostly bears a longitudinal stripe in the middle. Laterally, the larvae have a subspiracular wide white band along the body. The setigerous tubercles are conical, larger in the abdominal segments I, II and VIII, with all tubercles bearing a seta and several very tiny spicules. The head capsule is ochre to brown (Figure 2.22 B, Figure 2.23 A-C). Length of the mature larvae ranges from 25.5 to 36.0 mm (Crumb, 1956; Angulo et al., 2006; Sánchez & Sánchez, 2008).

Pupae are obtect, shiny reddish brown to dark brown as they are closer to the emergence of the adults. Length of the pupae is 15 – 18 mm and width averages 4.7 mm (Carrera, 2013; Capinera, 2020).

Adults are yellowish brown, slightly tinged with green. Fore wings bear three oblique brown bands, each accompanied with a whitish band (Figure 2.22 C); hind wings are whitish, darker near the external margin. Females tend to be darker than males. Wingspan is 28 - 35 mm (Carrera, 2013; Capinera, 2020).

An identification key is given by Possoa (2014) and a detailed morphological description by Carrera (2013).

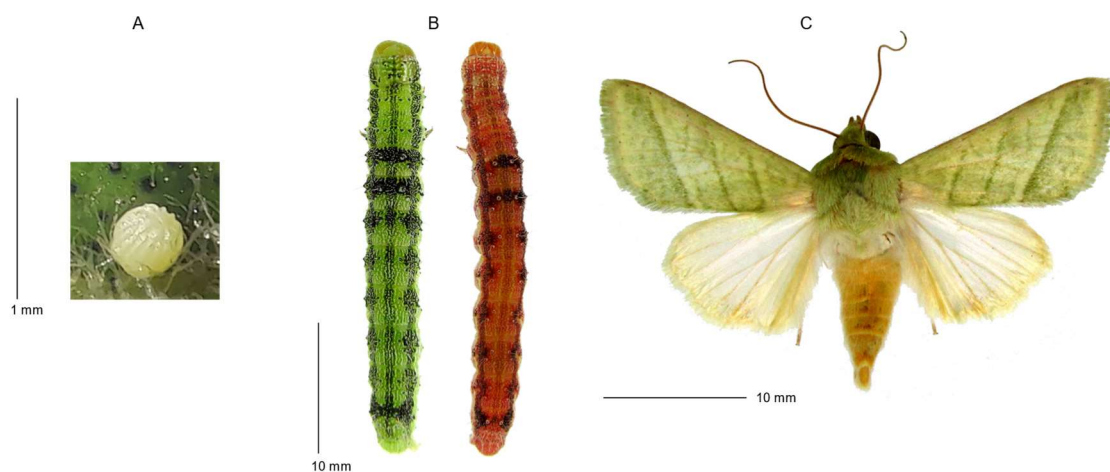


Figure 2.22. Life stages of *C. virescens*: A. Egg; B. Larvae; C. Adult (Photos: L. Cruces, Y. Callohuari).

2.8.8.4. Life cycle

Under laboratory conditions (25 ± 1 °C, 70% RH), the egg incubation period averages 3.5 days, larval development with asparagus as food takes from 23 to 46 days while the pupal period takes 12 to 17 days. Adult longevity is from 41.5 to 58.5 days. The fecundity of the females ranges

between 346 and 1021 eggs (Carrera, 2013), although Fye & McAda (1972) reported up to 1500 eggs per female.

2.8.8.5. Host plants

This species has a wide range of hosts, which usually are members of the families Amaranthaceae, Alliaceae, Asteraceae, Euphorbiaceae, Geraniaceae, Fabaceae, Liliaceae, Malvaceae, Nyctaginaceae, Pedaliaceae, Poaceae, Solanaceae, Rosaceae and Vitaceae. In Peru it infests crops such as quinoa, cotton, asparagus, artichoke, beans, apple, blueberry, and chickpea (*Cicer arietinum* L.) (Sánchez & Sarmiento, 2002; Sánchez & Vergara, 2003; Molina-Ochoa et al., 2010).

2.8.8.6. Behaviour and crop damage

Adults have a nocturnal behaviour. Females lay single eggs on flowers, reproductive organs and terminal growth. Larvae of the first instar scrape on the surface of the leaves while later instars infest the panicle to feed on developing grains (Figure 2.23 A-C). When the larval stage is about to be complete, the larvae migrate down to the ground looking for refuge to pupate (Carrera et al., 2016; Capinera, 2020).

Damage by *C. virescens* on leaves is mostly not economically relevant, but the feeding on the grains does affect the quinoa production; harvested grains are usually contaminated with the larval feces (Figure 2.23 D) (Carrera et al., 2016).



Figure 2.23. Larvae of *C. virescens* infesting quinoa (A-C), and their damage in the panicle (D) (Photos: author).

2.8.9. *Spodoptera eridania* (Cramer)

2.8.9.1. Taxonomy

This species, commonly known as the southern armyworm, has the following classification (Lafontaine & Schmidt, 2010; Capinera, 2020):

CLASS :	Insecta
ORDER :	Lepidoptera
FAMILY:	Noctuidae
GENUS :	<i>Spodoptera</i>
SPECIES:	<i>eridania</i> (Cramer, 1784)

2.8.9.2. Distribution

This species is native to America, and occurs in North, Central and South America and the Caribbean (Pogue, 2002; Pastrana, 2004; Bentancourt & Scatoni, 2006; Angulo et al., 2008).

Although it is not established in Europe, an accidental introduction has been reported in Denmark (Karsholt, 1994).

2.8.9.3. Morphology

The eggs are light green, turning to grey as they mature. They are spherical but flattened at its base. Eggs are laid in clusters and covered with some scales from the body of the female (Figure 2.24 A). Diameter of the egg averages 0.45 mm and its height is 0.35 mm (Montezano et al., 2013).

First instar larvae are light to dark green, with lateral longitudinal yellow lines. More mature larvae are green or blackish green, bearing a narrow medial white line dorsally, and one stripe dorso-laterally at each side; a series of dark triangles dorso-laterally along the body length is often present (Figure 2.24 B). Laterally, the larvae bear a broad yellowish or whitish stripe, interrupted by a dark spot on the first abdominal segment (Figure 2.25 A). The ventral colouration is pinkish to orangish, bearing tiny white spots. The head is orange to reddish-brown. Length of the mature larvae averages 35 mm (Crumb, 1956; Capinera, 2020).

Pupae are obtect, mahogany brown and shiny. Their length is 16 to 18 mm and their width, 56 mm (Sánchez & Vergara, 2003; Capinera, 2020).

The adults are greyish brown (Figure 2.24 C). Fore wings have a variable marking pattern: some individuals bear a dark reniform spot near the centre of the wing; in other specimens, this spot is absent, and instead there is a broad black band which extends from the centre to the margin of the wing. Hind wings are white and opaque. Wingspan is 33-38 mm (Capinera, 2020).

Identification keys are given by Caballero (1994) and Pogue (2002).

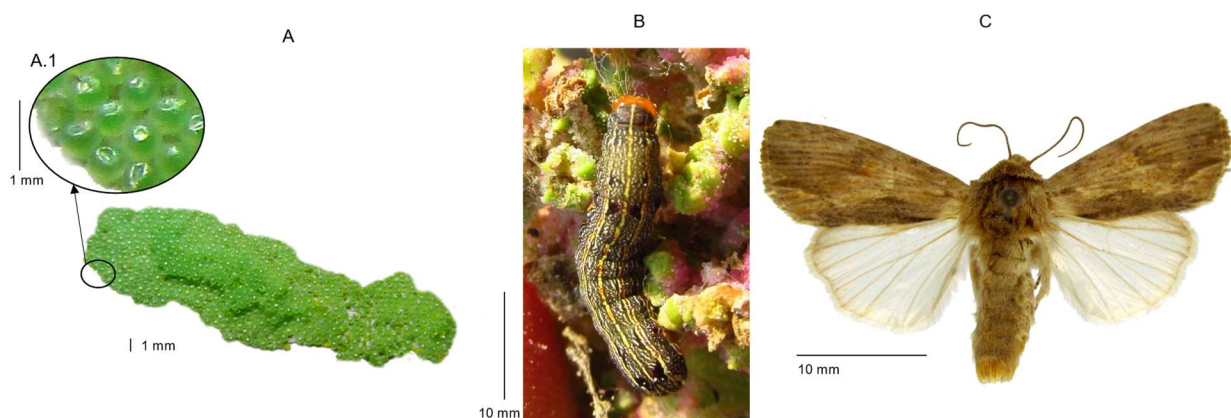


Figure 2.24. Life stages of *S. eridania*: A. Eggs, A.1 enlarged section; B. Larva; C. Adult (Photos: L. Cruces; Y. Callohuari).

2.8.9.4. Life cycle

Under laboratory conditions (25 ± 1 °C, $70 \pm 10\%$ RH, and a 14 h photoperiod), the eggs hatch in 4 days, larval development on an artificial diet averages 17.7 days, and the pupal period is 9.2 days. Female fecundity under these conditions ranges from 1848 to 2012 eggs (Goulard et al., 2014).

2.8.9.5. Host plants

This is a polyphagous pest, with at least 106 plants reported as host, 56 of them corresponding to crops. In Peru, *S. eridania* is an important pest in quinoa, tomato, asparagus, artichoke, kanihua, sweetpotato, broccoli (*Brassica oleracea* L.) and occurs in several other crops, weeds and wild plants of the family Amaranthaceae, Asteraceae, Brassicaceae, Convolvulaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Malvaceae, Phytolaccaceae, Poaceae, Polygonaceae, Rosaceae, Rubiaceae and Solanaceae (Sánchez & Vergara, 2003; Sánchez & Sánchez, 2008; Goulard et al., 2014).

2.8.9.6. Behaviour and crop damage

Adults have a nocturnal behaviour. Females lay their egg clusters, both on the upper side and the underside of the leaves, covered with a few scales (Sánchez & Vergara, 2003).

First instars are gregarious and feed by scratching tissue off the leaves. Later instars become more voracious, and they disperse over the different parts of the plant where they often skeletonize the leaves, causing a strong defoliation (Figure 2.25 A). At the grain filling stage, the larvae infest the panicle to feed on the developing grains, causing direct damage on quinoa production (Figure 2.25 B) (King & Saunders, 1984; Dos Santos et al., 2010; Carrera et al., 2016).

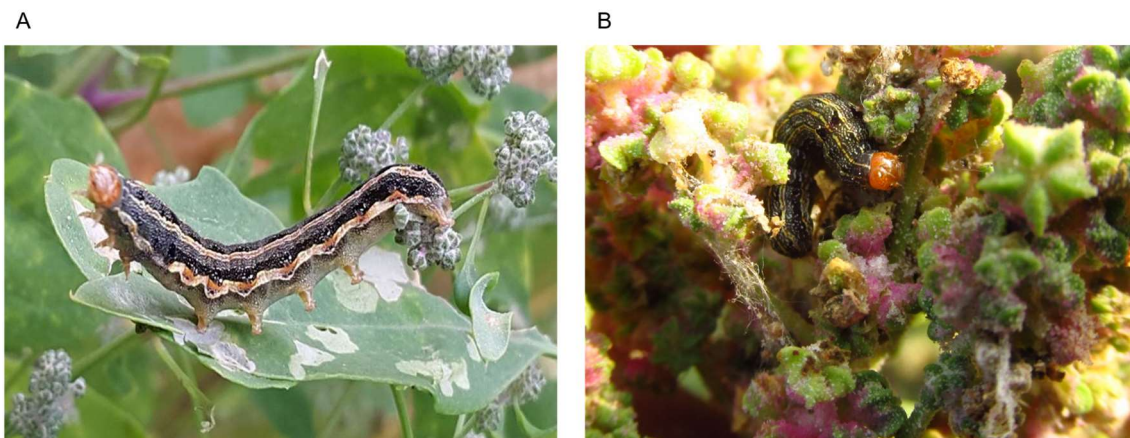


Figure 2.25. *S. eridania*: A. Larva and damage on the leaves; B. Larva on panicle (Photos: L. Gómez; L. Cruces).

2.8.10. Natural enemies of quinoa pests in Peru

2.8.10.1. Predators

There is often little published information on the predatory guild in quinoa fields in South America. Moreover, the literature mostly refers to reports of species observed in the crop, and barely documents their functional behaviour in the agroecosystem, mainly due to a lack of in depth studies on biological control agents of quinoa pests.

The main reported predatory insect groups belong to the orders Coleoptera, Dermaptera, Diptera, Hemiptera and Neuroptera; spiders, members of the order Araneae are also significant in quinoa (Saravia et al., 2014; Valoy et al., 2015; Cruces et al., 2016; Chapter 4). Below, the predatory species reported in Peru are listed, according to the taxonomic group they belong to. Species reported in the Altiplano of Bolivia are also included, since a similar natural enemy complex in the Peruvian Altiplano may occur.

a. Coleoptera

The most common predatory coleopterans in quinoa are ground beetles and ladybirds, and to a lesser degree rove beetles.

The ground beetles are members of the family Carabidae. The adults are mainly nocturnal and they are very common in agricultural systems (Triplehorn & Johnson, 2005; Menalled & Landis, 2008; Riddick, 2008). Larvae and adults of ground beetles feed on epigeous insects such as cutworms, crickets and caterpillars that go down from the foliage to the soil to pupate (as do many noctuids) (Sánchez & Vergara, 2005). In quinoa in the Altiplano of Peru the following species are reported: *Notiobia schnusei* Van Emden, *N. laevi bolivianus* Van Emden and *Meotachys* sp. (Valoy et al., 2015). In the Altiplano of Bolivia, besides the above species, there are also reports on *Bembidion quadrimaculatum* (Linnaeus), *Stenolophus plebejus* Dejean, *Chlaenius sericeus* (Forster), *Chlaenius* sp., *Amara* sp., and *Pterostichus* sp. (Saravia et al., 2014). In the coastal areas of Peru, the most frequent species collected in quinoa is *Blennidus peruvianus* (Dejean) (Figure 2.26 A) (Cruces et al., 2016; Soca, 2021; Chapter 4).

Ladybirds are members of the family Coccinellidae. Larvae and adults are avid predators of aphids and others small soft-bodied insects. In Peruvian quinoa from the highlands, *Hippodamia convergens* Guérin-Ménéville and *Eriopis connexa connexa* (Germar) have been noted, whereas for the coastal areas the following species have additionally been recorded: *Cycloneda sanguinea* (Linnaeus), *Cycloneda* sp., *Scymnus ocellatus* Blackburn & Sharp, *Eriopis peruviana* Hofmann, *Paraneda pallidula guticollis* (Mulsant), and *Harmonia axyridis* Pallas (Figure 2.26 B-D) (Cruces et al., 2016; Chapter 4).

The rove beetles are members of the family Staphylinidae. Predatory species feed on ground dwelling prey. In Bolivian quinoa *Homeotarsus* spp. have been noted to occur but there is little other published information (Saravia et al., 2014).

b. Dermaptera

Earwigs of the family Labiduridae are predacious species that feed on soil surface dwelling arthropods. In Peruvian quinoa fields *Labidura riparia* (Pallas) has been recorded in coastal areas (Cruces et al., 2016).

c. Diptera

The most common predatory dipteran species in quinoa are the hoverflies and the long-legged flies, and to a lesser degree robber flies have also been reported.

Hoverflies belong to the family Syrphidae. The predatory syrphid larvae commonly feed on aphids whereas adults feed on nectar and pollen. In quinoa in the coastal areas of Peru the following species have been observed: *Allograpta exotica* Wiedemann, *A. peruana* Shannon, *Pseudodoros clavatus* (Fabricius), *Syrphus shorae* Fluke and *Toxomerus* sp. (Figure 2.26 E) (Cruces et al., 2016; Soca, 2021; Chapter 4).

Long-legged flies are insects of the family Dolichopodidae. Adults feed on small insects and mites. *Condylostylus quadricolor* (Walker) and *Chrysotus* sp. have been found in quinoa fields at the Peruvian coast (Figure 2.26 F) (Cruces et al., 2016; Soca, 2021; Chapter 4).

Robber flies are members of the family Asilidae. Adults feed on a variety of relatively large insects. In Bolivian quinoa, *Erax* sp. has been reported (Saravia et al., 2014).

d. Hemiptera

Predatory hemipteran species belong to the sub-order Heteroptera, within the families Anthocoridae, Berytidae, Geocoridae, Nabidae and Pentatomidae.

Anthocorids, berytids, geocorids and nabids feed on small insects, mainly of the suborder Sternorrhyncha (e.g. aphids) but also on other small insects such as thrips, the eggs or first instars of lepidopterans, and mites. Predatory pentatomids usually feed on lepidopteran larvae (Cruces et al., 2016).

In the quinoa fields of the coastal areas of Peru, the following species have been reported: *Orius tricolor* (White), *O. insidiosus* (Say), *Paratriphleps laeviusculus* Champion (Anthocoridae), *Rhinacloa* sp. (Miridae), *Metacanthus tenellus* Stål (Berytidae), *Geocoris* sp. (Geocoridae), *Nabis*

capsiformis Germar (Nabidae), and *Podisus nigrispinus* (Dallas) (Pentatomidae)(Figure 2.26 G, H) (Valoy et al., 2015; Cruces et al., 2016; Soca, 2021; Chapter 4).

e. Neuroptera

The most common neuropteran species are the green and brown lacewings.

Green lacewings belong to the family Chrysopidae. Larvae are important predators of small soft-bodied insect such as aphids, whiteflies, thrips, eggs and small larvae of lepidopterans. Adults feed on nectar and pollen. *Chrysoperla externa* (Hagen) has been found in quinoa of the Peruvian coastal areas (Figure 2.26 I) (Valoy et al., 2015; Soca, 2021).

Brown lacewings belong to the family Hemerobiidae. Larvae and adults also are important predators of small soft-bodied insects and mites. *Symphorobius* sp. and *Hemerobius* sp. have been found to occur in quinoa in the lowlands of Peru (Cruces et al., 2016; Soca, 2021).

f. Araneae

Spiders are very abundant in quinoa fields. The most common species that inhabit the crop in Peru belong to the following families: Thomisidae, Oxyopidae, Lycosidae, Dysderidae and Salticidae (Cruces et al., 2016).

2.8.10.2. Parasitoids

The main groups of parasitoids belong to the orders Diptera and Hymenoptera.

The most common dipteran parasitoids belong to the family Tachinidae, which parasitize lepidopteran larvae and to a lesser degree, heteropteran nymphs. Hymenopteran parasitoids commonly found in quinoa in South America belong to the families Braconidae, Ichneumonidae, Scelionidae, Pteromalidae, Eulophidae and Trichogrammatidae. Species of these families are oligophagous, with relatively specific host species and stages (Figure 2.27 A-D)(Saravia et al., 2014; Cruces et al., 2016).

Like for the predatory guild, there is little published information in South America about the interactions between quinoa, its herbivores and their parasitoids and most of the observations on insect parasitoids observed in quinoa fields refer to the key pest *E. melanocampta*. Annex 4 compiles the reported parasitoids from Peru whose host range includes pests of quinoa from the Andean and Coastal regions (Saravia et al., 2014; Valoy et al., 2015; Cruces et al., 2016; Cruces et al, 2020a, 2020b; Soca, 2021).

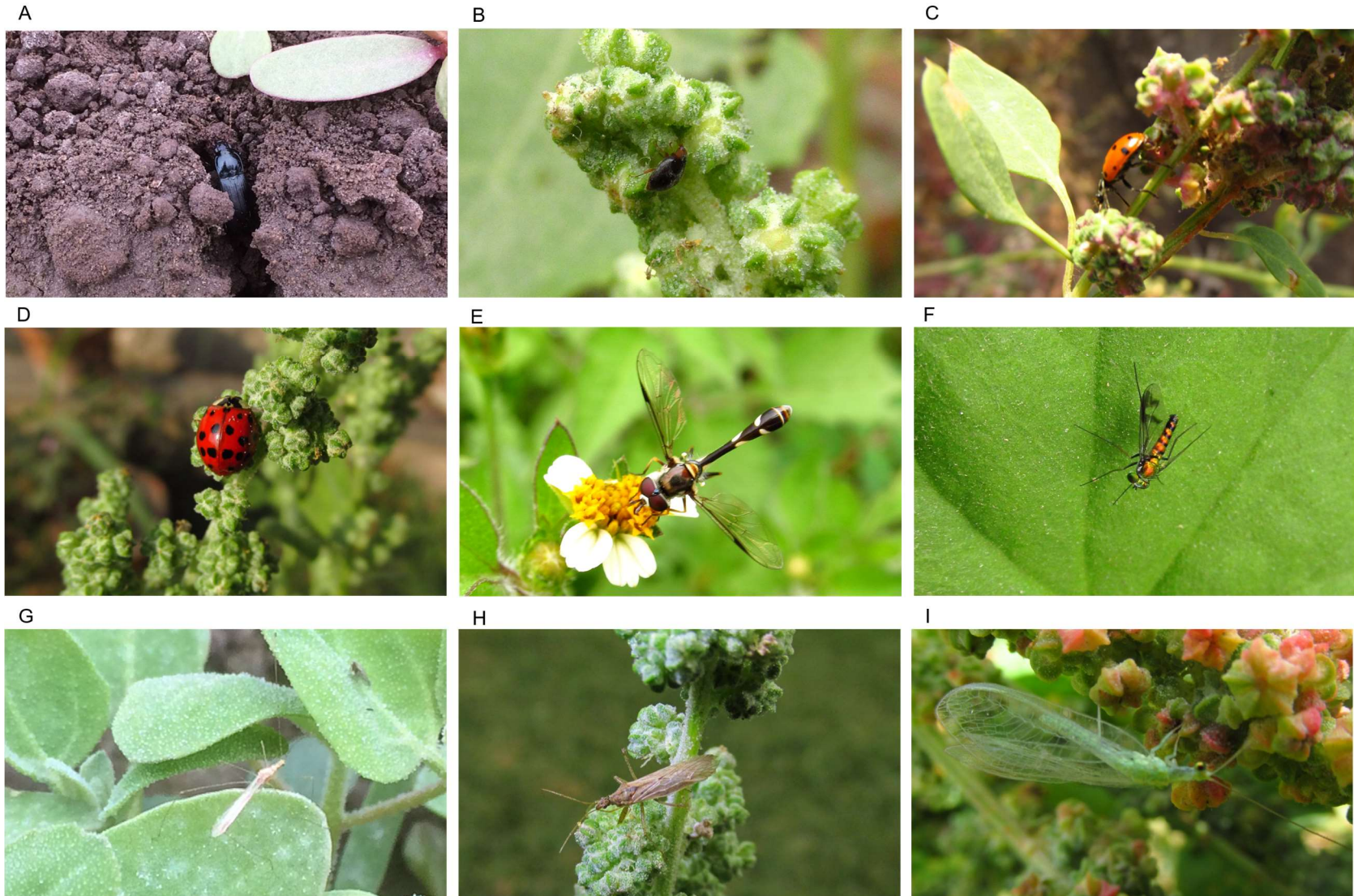


Figure 2.26. Diversity of predatory insects in quinoa. A. *B. peruvianus* (Carabidae); B. *S. ocellatus*; C. *H. convergens*; D. *H. axyridis* (Coccinellidae); E. *P. clavatus* (Syrphidae); F. *C. quadricolor* (Dolichopodidae); G. *M. tenellus* (Berytidae); H. *N. capsiformis* (Nabidae); I. *Chrysoperla* sp. (Chrysopidae) (Photos: author).

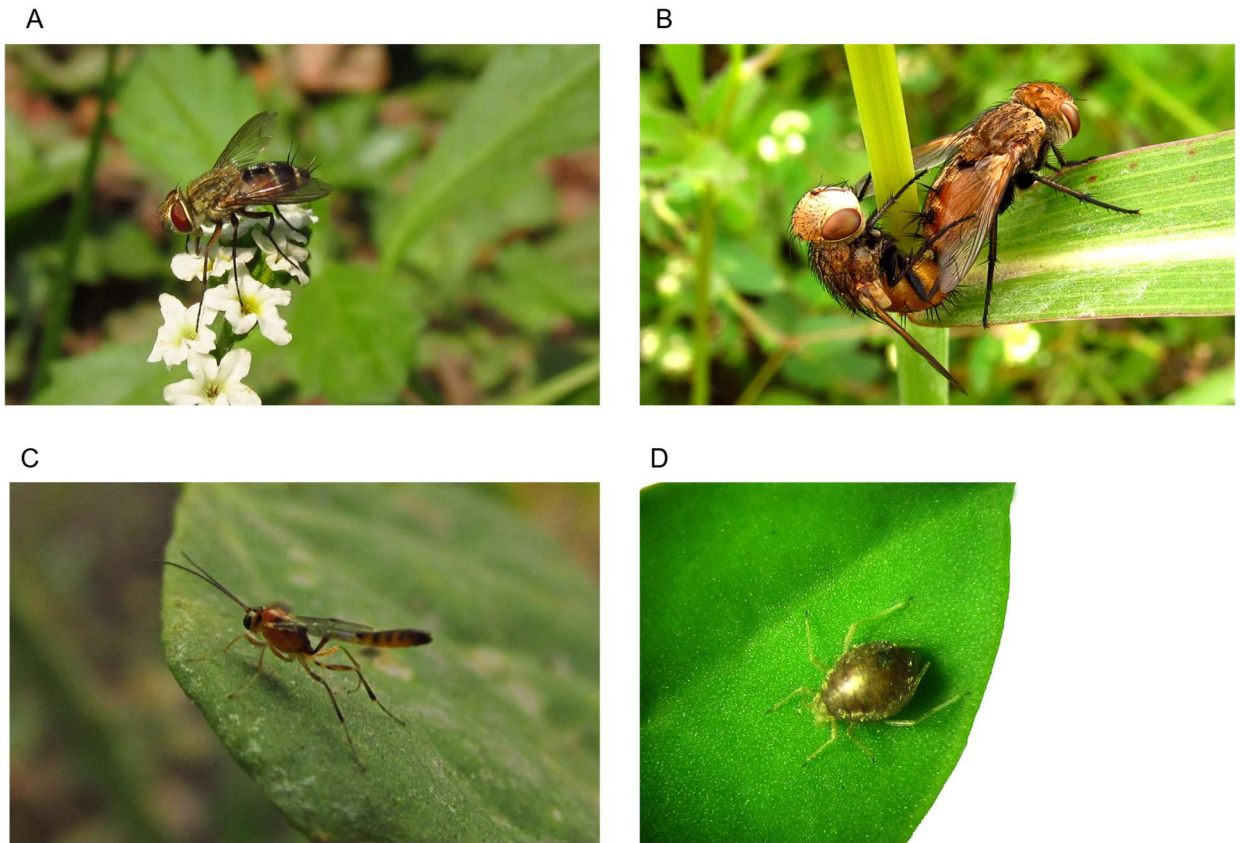


Figure 2.27. Parasitoids in quinoa fields: A and B. Tachinid species; C. An Ichneumonidae species; D. Mummified aphid (Photos: Y. Callohuari; L. Cruces).

2.9. Strategies of pest management in quinoa

As quinoa is a new crop in the lowlands of Peru, published information on pest management from these areas is relatively scarce so far. The information gathered below includes strategies applied in the Andes of Bolivia, which can serve as a reference when implementing IPM programs in the Andes of Peru and at lower elevations.

2.9.1. Monitoring and economic thresholds

2.9.1.1. Plant sampling

To monitor the pests in Peruvian quinoa fields, Cruces et al. (2016) recommend stratifying the field into 5 sectors and to sample at least 25 quinoa plants per 1 ha (5 per sector), whereas Saravia et al. (2014) recommended 10 plants per ha for quinoa cultivated in the Andean region of Bolivia. Monitoring should be systematic throughout the crop phenology, but particular attention should be paid from flowering to grain maturation. Quinoa plants from the borders should always be avoided for sampling, because they are near to roads, ditches and nearby crops, and therefore they are not representative of the situation within the field (Quispe et al., 2014; Cruces et al., 2016).

The sampling units to take into consideration during the samplings are the following (Cruces et al., 2016):

- Whole plant (at germination), to monitor initial damage of leafminer fly adults.
- Leaves (during the vegetative stage), to monitor leafminer flies, aphids, and lepidopteran larvae.
- Flower primordia and leaves (during beginning of flowering), to monitor lepidopteran larvae and leafminer flies.
- Whole panicle (from flowering to maturation), to monitor lepidopteran larvae and thrips.

2.9.1.2. Use of traps

The use of coloured sticky traps, molasses traps and pheromone traps has been incorporated in the pest management in quinoa, albeit not extensively.

a. Colour and molasses traps

Coloured sticky traps have been used in quinoa in the coastal areas of Peru. These traps are placed in the borders of the field (Figure 2.28, 2.29), and they are aimed mainly to monitor and trap adults of leafminer flies, thrips, aphids and heteropterans. Due to the structure of the

quinoa plants and the high plant density used, mass trapping has not been implemented and such traps are thus mainly employed for monitoring purposes (Cruces et al., 2016).



Figure 2.28. Yellow sticky trap in the border of a quinoa field.

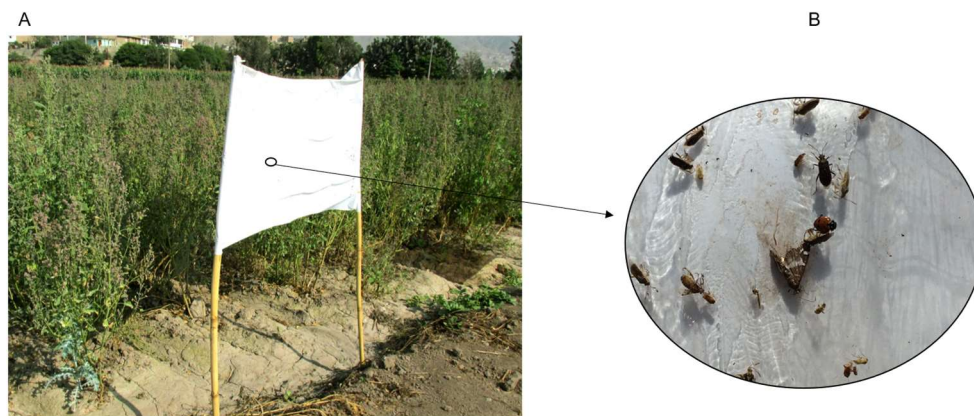


Figure 2.29. A. White sticky trap in a quinoa field. B. Enlarged section.

Molasses traps have also been included to monitor pests, particularly adults of Lepidoptera. Such traps are handcrafted by recycling bottles and containers of different sizes, using molasses and water in a proportion of 1:1. Lepidopteran pests, such as *S. eridania*, *C. virescens*, *S. recurvalis* and *E. melanocampta*, are attracted by the sweet odour of the molasses and they are captured by its sticky consistence (Figure 2.30, 2.31). However, other non-pest lepidopterans can also be attracted, and the evaluator may confuse them with the quinoa pests; more in particular the smallest moths may be difficult to distinguish from *E. melanocampta* which easily lose their wing scales when trapped. Therefore, this kind of traps is more useful to monitor larger-sized moths such as noctuids and some pyralids (Cruces et al., 2016).



Figure 2.30. Bottle molasses trap in a quinoa field.

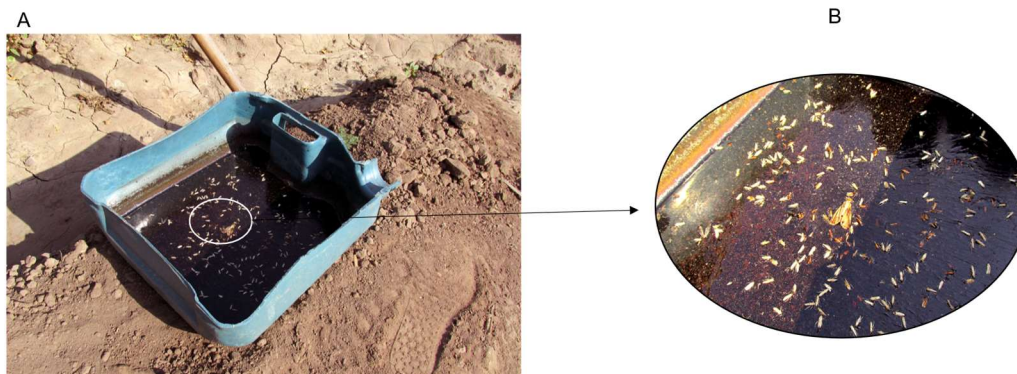


Figure 2.31. A. Molasses trap from a recycled container. B. Enlarged section.

b. Pheromone traps

In Bolivia, experiences with the use of pheromone traps have been reported for noctuid moths, with commercially available synthetic pheromones for *H. quinoa*, *C. incommoda* and *Agrotis andina* Köhler. Four pheromone traps per hectare are recommended to monitor the presence of the adults of these species. In zones where population densities of these noctuids are low, the use of 4 pheromone traps per hectare allowed to keep larval damage below economic levels (Saravia et al., 2014).

The use of pheromone traps in the lowlands of Peru to monitor noctuids such as *C. virescens* or *S. eridania*, has not been incorporated in the pest management in quinoa, although synthetic pheromones for *C. virescens* have recently been made commercially available in Peru (SENASA, 2022).

2.9.1.3. Economic thresholds

Economic thresholds have been defined for a very limited number of quinoa pests. When numbers of *H. quinoa* larvae average more than 1 individual per plant, Saravia et al. (2014) recommend applying control methods, whereas Quispe et al. (2014) suggest an economic

threshold level of 3-6 larvae per plant for *E. melanocampta* or *E. quinoa*. Economic thresholds for quinoa production in the lowlands have not been reported.

2.9.2. Cultural practices

2.9.2.1. Soil preparation

Either through traditional practices (in the Andes) or by using machinery (at lowlands), soil preparation contributes to reduce cutworms and other pests that pupate in the soil (i.e. some lepidopterans and dipterans). In the coastal areas, farmers usually perform irrigation before soil preparation, in order to facilitate the use of tillage tools; this practice also contributes to eliminate remaining pest stages from the previous crop and promotes the germination of weeds that later are destroyed during the tillage (Saravia et al., 2014; Gómez & Aguilar, 2016; Cruces et al., 2016).

2.9.2.2. Plant density

Usually, quinoa is manually sown, aided with perforated small containers that allow the seed to pass through, using more seeds than necessary (10-20 kg/ha); this results in a high density of germinated plants. This occurs mainly due to the limited access to sowing machines, which significantly reduce the amount of seed used at sowing (4-5 kg/ha). However, this high stand density of quinoa allows the farmers to overcome initial damage caused by cutworms, and about two weeks after germination they eliminate the excess of plants in order to obtain a suitable plant density, depending on the variety used (Cruces et al., 2016; Gómez & Aguilar, 2016).

2.9.2.3. Sowing and fallow periods

Under Andean conditions, quinoa is rainfed irrigated, therefore the sowing period takes place when the raining season begins. Thus, the fallow periods correspond to the dry months when farmers cannot cultivate any crops. In this way the life cycle of insect pests can be broken and soil fertility is not depleted (Gómez-Pando et al., 2014; Saravia et al., 2014).

2.9.2.4. Cultivation and hilling

Either manually (in the highlands) or by using machinery (in the lowlands), cultivation and hilling are common practices in quinoa, aimed at improving aeration in the soil to the roots and providing a better support to the base of plants. With these practices, larvae and pupae of pests, alongside weeds, are suppressed (Bazile et al., 2014).

2.9.2.5. Weed control

Most of the weeds that infest quinoa correspond to species of the family Amaranthaceae (the same family as that of the crop), which serve as a source of food and refuge for the phytophagous insects that feed on quinoa. These weeds, and others of different families, are controlled during the tilling, cultivation and hilling practices. However, manual weeding is also done in order to reduce weed density, mainly 2-3 weeks after germination (Figure 2.32) and during the flowering stage (Cruces et al., 2016; Gómez & Aguilar, 2016).

Currently, there are no specific herbicides used in quinoa in Peru. The systemic herbicide glyphosate may be applied, but only in the field borders and near irrigation ditches (Gómez & Aguilar, 2016).



Figure 2.32 Manual control of weeds.

2.9.2.6. Crop rotation and intercropping

Farmers from the highlands usually consider other Andean crops which have long been part of their traditional staple crops. These include native tubers (e.g. potato, oca, maca), native grains, corn and a range of legumes (e.g. broad bean, tarwi). This practice allows to interrupt the life cycle certain of insect pests and increases the benefits of biodiversity in the agroecosystem (Altieri & Nicholls, 2004; Thrupp, 2004; Ramani, 2013; Gómez-Pando et al., 2014; Saravia et al., 2014). In the traditional cultivation system, intercropping with these native crops is also incorporated as a cultural practice by farmers from the highlands.

2.9.3. Use of bioinsecticides

Environmentally friendly insecticides include microbial formulations, bacterial fermentation products, and botanicals. In Peru, *Bacillus thuringiensis kurstaki*, *B. thuringiensis aizawai* and

spinosad are sprayed against *S. eridania*, *E. melanocampta*, *S. recurvalis* and *C. virescens*. They are used to reduce lepidopteran larval densities in organic quinoa production. Tetraethyl silicate has been used against aphids. To enhance efficacy of these insecticides, the use of adjuvants is recommended (Saravia et al., 2014; Gómez & Aguilar, 2016; SENASA, 2022).

Although less extensively so, treatments with the entomopathogen *Beauveria bassiana* is also recommended to control *C. virescens* and *F. occidentalis*, particularly at the beginning of the infestation (Gómez & Aguilar, 2016; SENASA, 2022). Bioinsecticides based on granulosis and nucleopolyhedrosis viruses have been tested against *E. melanocampta*, with up to 50% of control (Valoy et al., 2015).

Another group of alternative compounds the use of which is rapidly increasing are insecticidal botanical extracts. In Peru, a number of botanical extract products have been registered, including: *Azadirachta indica* against *F. occidentalis*, *L. huidobrensis*, and *M. euphorbiae*; *Allium sativum* and *Capsicum annuum* against *N. simulans*; *Sophora flavescens* against *S. eridania*. (Gómez & Aguilar, 2016; SENASA, 2022). Moreover, the matrine type of alkaloids has recently been included in the control of aphids and thrips (SENASA, 2022).

The following plant species may be candidates for preparing extracts due to their reported insecticidal activity: *Chrysanthemum cinerariaefolium*, *Satureja parviflora*, *Baccharis incarum*, *Parastrephia lucida*, *Nicotiana tabacum*, *Schinus molle* and *Polylepis incana*. However, their registration in SENASA (National Service of Agrarian Sanitation) is needed for wide scale use (Gómez & Aguilar, 2016; SENASA, 2022).

2.9.4. Use of synthetic chemical insecticides

In conventional quinoa production, farmers usually rely on broad spectrum insecticides such as pyrethroids (i.e. cypermethrin, alphacypermethrin, lambda-cyhalothrin), organophosphates (chlorpyrifos, methamidophos, dimethoate, monocrotophos) and carbamates (carbaryl, cartap, methomyl) (Saravia et al., 2014; Gómez & Aguilar, 2016; Latorre, 2017; Chapter 4). Moreover, these pesticides are mostly used without a rotation system and sometimes, they are applied following a fixed schedule of treatments (i.e. calendar spraying) rather than a system based on the infestation level; this is done up to 60 days after germination, in order to reduce the risks of harvests being contaminated with chemical residues (Latorre, 2017; Chapter 4).

There is a wide variety of more selective insecticides with less harmful effects to humans and non-target organisms in the environment available on the Peruvian market. Compounds like

emamectin benzoate, lufenuron, spinosad, teflubenzuron, and clorantraniliprole may be part of the pest management under a rotation system of pesticides, based on their mode of action (Gómez-Pando et al., 2014; Saravia et al., 2014; Sparks & Nauen, 2015; SENASA, 2022; Cruces et al., 2021).

2.10. Integrated Pest Management (IPM)

2.10.1. Definition

IPM refers to coordinated strategies of pest management in order to keep pest populations at levels below economic thresholds. An IPM approach takes advantage of the factors of natural mortality, complemented with applied methods of pest control (biological, chemical, physical and cultural management strategies), considering the least possible disruption to agroecosystems and as minimal risks as possible to humans and the environment (Sánchez, 2006; Peshin & Zhang, 2014; Reddy, 2014; FAO, 2022).

2.10.2. Components of an IPM program

Four basic components can be identified when setting up IPM programs (Reddy, 2014):

1. *Planning*

Many pest strategies require steps or inputs which should be considered before planting, well in advance. Some considerations include the following: planning of crop rotations taking into account the implications of the subsequent crops, choice of cultivars, technical information of seeds, monitoring options (equipment, personal), and specialized equipment necessary for the crops.

2. *Pest identification*

Only with a correct identification of the pests, appropriate methods of monitoring and pest control can be applied. Accurate identification of the pest may open a broad window of knowledge including plant hosts and non-hosts, plant parts that are attacked, refuges, preferred sites for oviposition, time of pest peak populations during the cropping season, possible presence of natural enemies, etc.

3. *Monitoring*

Once the pests are identified, they and their natural enemies must be systematically monitored throughout the crop phenology. An appropriate sampling technique should be considered in the planning step. Tracking the pest populations will allow to identify

the time when densities approach the economically damaging levels thus allowing suitable methods of pest control to be timely applied.

4. *Economic injury and action levels*

The economic injury level (EIL) is “the lowest population density of a pest that will cause economic damage, or the amount of pest injury which will justify the cost of control” (Stern et al., 1959; Cisneros, 2012). In an IPM approach, farmers use the concept of economic threshold level (ETL), also known as an action threshold, which refers to the point at which pest control methods should be applied to prevent that the pest population reaches injurious levels.

Chapter 3

Insect diversity associated with quinoa (*Chenopodium quinoa* Willd.) in three altitudinal production zones of Peru

Redrafted after:

Cruces, L., de la Peña, E., and De Clercq, P. 2020. Insect diversity associated with quinoa (*Chenopodium quinoa* Willd.) in three altitudinal production zones of Peru. *International Journal of Tropical Insect Science*, 40(4), 955-968.

Author Contributions:

Conceptualization, L.C., E.d.I.P. and P.D.C.; methodology, L.C. and P.D.C.; formal analysis, L.C., E.d.I.P. and P.D.C.; investigation, L.C., E.d.I.P. and P.D.C.; data curation, L.C.; writing—original draft preparation, L.C.; writing—review and editing, L.C., E.d.I.P. and P.D.C.; supervision, E.d.I.P. and P.D.C.

3.1 Introduction

Quinoa (*Chenopodium quinoa* Willd.) is cultivated in the Andean region of Argentina, Bolivia, Chile, Ecuador and Peru since ancestral times (Saravia et al., 2014; Gómez & Aguilar, 2016). The increasing popularity and market value of this crop have motivated more farmers to grow quinoa in several South American countries which has led to a considerable evolution of the planted area (Cruces et al., 2016). Moreover, different countries worldwide are increasingly interested in growing this grain (Bazile et al., 2014).

In Peru quinoa is traditionally cultivated in the highlands, in the departments of Apurímac, Ayacucho, Cusco, Huancavelica, Junín and Puno which cover about 90% of the planted area of this crop (Albújar, 2017). In the last decade, new quinoa production zones have emerged at lower altitudes such as the Arequipa region at 1,410 m a.s.l., and the Lambayeque, La Libertad, Lima, Piura and Tacna departments at coastal level (OEEE, 2012; Sifuentes et al., 2016; Albújar, 2017).

Exploration of new areas for cultivating quinoa must go hand in hand with an assessment of possible plant health risks and the implications of agricultural management on the biodiversity, especially at low altitudes where this Andean grain has not been traditionally cultivated. With the expansion of quinoa production to these low altitude areas, new pest problems are being reported (Cruces et al., 2016). Moreover, there is limited documentation from these areas about the phenology of the pests of quinoa and even less information about their natural enemies. Consequently, inappropriate pest management practices are being applied (e.g. the lack of crop rotation, the use of broad spectrum insecticides) (Cruces et al., 2016; Latorre, 2017), which affect the local insect diversity as reflected in a reduction of the species richness and skewed relative abundance distributions (Tylianakis et al., 2007). Negative effects on the beneficial entomofauna may eventually weaken the biological control function over the herbivorous community, causing further pest outbreaks (Crowder & Jabbour, 2014).

The soil surface and lower crop strata of an agroecosystem reflect a representative part of the total composition of the insect community, as this ecological niche is in permanent interaction with higher crop strata. For example, certain species migrate from the plant to the soil for pupation; some moths remain hidden in the lower parts of the crop during the day before becoming active at night; and, some small insects such as psyllids, thrips and aphids, easily drop from the plant by wind, rain or other factors (Sánchez & Vergara, 2002). Consequently, pitfall trapping can constitute an effective methodology to analyse the diversity of agroecosystems, including that of plant-dwelling species (Gill & McSorley, 2012); besides, pitfall traps are also

used to infer differences in population size of species between sites, especially when they are used for long periods of time (i.e. more than four months) (Leather, 2005).

Given the increasing expansion of quinoa to new production areas and its possible consequences mentioned above, it is worth investigating to what extent the insect communities that inhabit the crop in the newly exploited areas differ from those of the traditional production areas. The main objective of this study was to describe the differences between the three localities with regard to their entomofauna associated with quinoa in terms of composition, species diversity and functional diversity between and within the localities (in terms of species evenness and species richness). This contribution aimed at shedding light on the consequences of farming intensification and expansion of quinoa on the local insect diversity in Peru. The study therefore provides new insights for applying integrated pest management (IPM) in the cultivation of this crop.

3.2. Materials and Methods

3.2.1. Data collection

The study was carried out in three altitudinal production zones of Peru: (i) La Molina (244 m a.s.l.), located in one of the most recent areas used for quinoa production on the coast, Lima; (ii) Majes (1,410 m a.s.l.), in the natural region called “Maritime Yunga” (Pulgar Vidal, 1981), in the department of Arequipa, situated between the eastern part of the coastal strip and the western part of the highlands, a zone that over the last years experienced an increase in the cultivated surface area for quinoa; and (iii) San Lorenzo (3,322 m a.s.l.), in the department of Junín, representing the traditional Andean localities for quinoa production where the crop has been grown for centuries (Gómez & Aguilar, 2016). The field sites located in La Molina and San Lorenzo were in experimental and production fields belonging the National Agrarian University La Molina, whereas the field site assessed in Majes belonged to a private farmer (see Annex 2,3).

The monitored fields were cultivated under conventional farming practices (with use of chemical pesticides) (Table 3.1). The cultivation and pest management specifications listed in Table 3.1 were given by the responsible of the field sites in La Molina and San Lorenzo (L. Gómez, personal communication, June 15, 2016) and in Majes (E. Falconi, personal communication, February 2, 2017). Meteorological data were provided by the weather station “Von Humboldt” at the National Agrarian University La Molina, the weather station Map-Pampa de Majes of the National Service of Meteorology and Hydrology of Peru (SENAMHI), and the weather station at the Regional Institute of Highland Development in Jauja of the National Agrarian University La

Molina. Geographical data was obtained from the literature (Takano & Castro, 2007; Vilca et al., 2015; Núñez, 2016).

The insect community associated with the quinoa crop was examined with pitfall traps (transparent, \varnothing 10 cm at opening and at bottom, 10% ethylene glycol, water and detergent) (Leather, 2005), which were left during the whole crop phenology (for La Molina, from 08/09/2015 to 29/12/2015; for San Lorenzo, from 11/01/2016 to 12/05/2016; for Majes, from 15/05/2016 to 23/09/2016). The pitfall trap content was periodically collected in airtight containers (16, 10 and 11 times for La Molina, San Lorenzo and Majes, respectively) and carefully labelled to be transported to the laboratory for further processing; the collection fluid was replaced every time. The sampling campaign was performed considering the planting season for each location. To avoid effects of rainfall, a small panel was placed above each trap at a height of 50 cm, at the field site in San Lorenzo.

At each locality, the field was divided into 5 strata (considering the slope of the field and the irrigation block); two traps separated by 10 furrows (8.5 m) were installed per stratum. The pitfall traps were then placed in the middle of each sector, being separated from other sectors by more than 25 meters (Leather, 2005) (Figure 3.1).

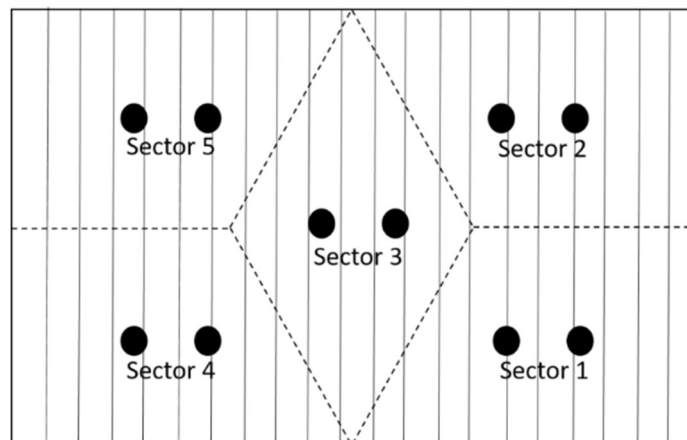


Figure 3.1. Diagram of the sectorization of the monitored fields and localization of the pitfall traps at all field sites under study. Black spots represent the pitfall traps; transversal lines represent the direction of the furrows.

Table 3.1. Growing specifications for quinoa during the sampling period in the localities of La Molina, Majes and San Lorenzo (Peru).

	Localities		
	La Molina District, Province of Lima, Department of Lima	Majes District, Province of Caylloma, Department of Arequipa	San Lorenzo District, Province of Jauja, Department of Junín
Mean monthly temp. (minimum–maximum)	16.67–22.97 °C	10.52–25.52 °C	6.96–20.06 °C
Mean monthly RH (minimum– maximum)	74.65–96.25%	31.2–60.2%	65.51–75.75%
Total precipitation during the sampling period	5.9 mm	0 mm	276.2 mm
Sowing–harvest	02/09/2015–10/01/2016	15/05/2016–20/09/2016	11/01/2016–20/05/2016
Field dimensions	85 m × 96.3 m (0.66 ha)	93.5 m × 96.3 m (0.9 ha)	102 m × 96 m (0.98 ha)
Variety	Pasancalla	Inia Salcedo	Pasancalla
Irrigation	Surface irrigation 100 irrigation furrows of 85 cm width, 10 irrigation blocks	Drip irrigation 110 irrigation furrows of 85 cm width, 4 irrigation blocks	Rain-fed 120 furrows of 85 cm width, 12 irrigation blocks
Soil type	Clay loam	Loamy sand	Loam
Neighbouring crops	Quinoa (<i>Chenopodium quinoa</i>); barley (<i>Hordeum vulgare</i>); kiwicha (<i>Amaranthus caudatus</i>); wheat (<i>Triticum</i> spp.)	Quinoa (<i>Chenopodium quinoa</i>); artichoke (<i>Cynara scolymus</i>)	Quinoa (<i>Chenopodium quinoa</i>); corn (<i>Zea mays</i>); potato (<i>Solanum tuberosum</i>)
Fungicides	1° benomyl (15/09/2015); 2° metalaxyl + mancozeb (04/10/2015); 3° dimethomorph (20/10/2015); 4° propamocarb + fluopicolide (03/11/2015)	1° benomyl (22/05/2016); 2° metalaxyl + mancozeb (12/06/2016); 3° dimethomorph (26/06/2016); 4° propamocarb + fluopicolide (10/07/2016)	1° benomyl (25/01/2016); 2° metalaxyl + mancozeb (14/02/2016); 3° dimethomorph (28/02/2016); 4° propamocarb + fluopicolide (15/03/2016)

Growing specifications for quinoa during the sampling period in the localities of La Molina, Majes and San Lorenzo (Peru) (continued).

	Localities		
	La Molina District, Province of Lima, Department of Lima	Majes District, Province of Caylloma, Department of Arequipa	San Lorenzo District, Province of Jauja, Department of Junín
Insecticides	1° <i>Bacillus thuringiensis</i> (27/10/2015); 2° dimethoate + methomyl (03/11/2015); 3° emamectin benzoate + methomyl (08/12/2015)	1° alpha-cypermethrin (22/05/2016); 2° emamectin benzoate (29/05/2016); 3° zeta-cypermethrin (12/06/2016); 4° alpha-cypermethrin (26/06/2016); 5° alpha-cypermethrin + emamectin benzoate (10/07/2016)	1° <i>Bacillus thuringiensis</i> + emamectin benzoate (04/04/2016)
Weed management	Manual control	Manual control	Manual control
Previous crop	Wheat	Corn	Fallow period of 6 months

Source for meteorological data: The weather station "Von Humboldt" at the National Agrarian University La Molina, the weather station Map-Pampa de Majes of the National Service of Meteorology and Hydrology of Peru (SENAMHI), and the weather station at the Regional Institute of Highland Development in Jauja of the National Agrarian University La Molina.

3.2.2. Sample processing and identification

All pitfall trap samples were processed at the laboratories of the Museum of Entomology “Klaus Raven Büller” of the National Agrarian University La Molina, in Lima, Peru, where the collected specimens were deposited.

The recipients containing pitfall trap samples were poured to a 1-mm mesh sieve and carefully washed with water, removing larger materials. The remaining sample (i.e. the collected insect specimens) were transferred to labelled glass vials (∅ 5 cm, 6 cm length) containing 75% v/v ethanol for conservation and further processing (i.e. morphotyping).

The samples were examined using a binocular stereoscope (Carl Zeiss: Stemi 508) and the specimens were sorted on the basis of morphological characteristics as morphospecies (Oliver & Beattie, 1993, 1996). Adults and nymphs were considered for paurometabolous insects, while for holometabolous species only adults were considered. Each new morphospecies was photographed and codified facilitating comparison when a new similar morphospecies was found; when necessary, the morphotypes were re-examined. Each morphotype was counted and registered according to the taxonomic group it belonged to and then preserved in a glass vial (∅ 1.5 cm, 2 cm length) with 75% v/v ethanol.

For identification purposes morphological descriptions and taxonomic keys provided in the literature were used to determine the families, subfamilies, tribes, genera and species (when feasible) of Coleoptera (Straneo, 1986; Moret, 1995; Arnett & Thomas, 2000; Arnett et al., 2002; Navarrete-Heredia et al., 2002; Moret, 2003; Bousquet, 2010; Biondi & D’Alessandro, 2012); Diptera (Spencer, 1973; Brown et al., 2009, 2010; Korytkowski, 2014); Hemiptera (Göllner-Scheiding, 1976; Hodkinson & White, 1979; Burckhardt, 1987a, 1987b, 1988, 1994; Blackman & Eastop, 2000, 2006; Triplehorn & Johnson, 2005; Cornelis et al., 2012; Henry et al., 2015; Panizzi & Grazia, 2015; Serbina et al., 2015; Pall et al., 2016; Horton et al., 2018); Hymenoptera (Masner, 1976; Bouček & Rasplus, 1991; Reina & La Salle, 2003; Fernández & Sharkey, 2006); Lepidoptera (Povolný, 1986); Neuroptera (Heckman, 2017); and Thysanoptera (Mound & Kibby, 1998).

Additionally, molecular tools were applied for identifying *Liriomyza huidobrensis* Blanchard, some Aphididae species, some *Aphidius* species, *Liorhyssus hyalinus* (Fabricius) and *Frankliniella occidentalis* (Pergande). DNA extraction and PCR procedures were performed in the Laboratory of Agrozoology, Department of Plants and Crops at Ghent University in Belgium, following the specific protocols provided in the literature (Shufran & Puterka, 2011; Derocles et al., 2012; Nakamura et al., 2013; Harbhajan & Kaur, 2017; T. Ding et al., 2018).

3.2.3. Statistical analysis

All statistical analyses were performed using R software, version 3.4.2 (R Core Team, 2020) (Packages: *vegan*, *RVAideMemoire*, *agricolae*, *biodiversity*, *MASS*) (Hervé, 2018; Kindt, 2018; Oksanen, 2009; Oksanen et al., 2020; De Mendiburu, 2020; Ripley et al., 2020).

The alpha diversity at each field site was analysed through a) the structure of the community with rank abundance curves, the Shannon index to measure the species evenness and the Simpson' dominance index to evaluate whether certain taxa dominate in the insect community, and b) the species richness with the Margalef index. These indices were also applied to assess the diversity of within feeding guilds (phytophagous and natural enemy complex) between and within field sites.

Non-parametric tests were used to compare alpha diversity indices between and within localities, after having established that they did not meet the requirements for parametric tests (homoscedasticity and sample size sufficiently large to corroborate their normal distribution) (Corder & Foreman, 2009). The Kruskal-Wallis test was applied for the comparisons of alpha diversity and functional guilds among field sites. For each locality, the Mann-Whitney-Wilcoxon test was used to compare the diversity of herbivorous versus entomophagous insects (i.e. natural enemies).

The estimated species richness of the assessed agroecosystem at each locality was calculated with non-parametric methods. Since pitfall traps render throughout the crop phenology abundance data for the different taxa retrieved, the most appropriate estimators were used, i.e. Chao1, Chao2 and the Abundance-based Coverage (ACE) (Gotelli & Colwell, 2011). These estimators differ in the way the rare species, unique species, singletons and doubletons are tallied; the corresponding formulas used are given in Table 3.2 (Chazdon et al., 1998; Moreno & Halffter, 2000; Magurran, 2004). These estimators were obtained through the software "Estimate-S 9.1" (Colwell, 2013). These parameters were also used for building species accumulation curves. The sampling effort was calculated by comparing each estimated species richness with the observed species richness.

The beta diversity was evaluated with the Bray-Curtis dissimilarity index and NMDS (non-metric multidimensional scaling) using the presence and abundance of the morphospecies to detect distances between the field sites. Significant differences between localities were assessed using pairwise PerMANOVA test (999 permutations). SIMPER analysis was used to determine the most influential morphospecies that contributed to the differences between localities (999 permutations). All tests were analysed at a significance level of $\alpha = 0.05$.

Table 3.2. Formulas for non-parametric estimators of species richness applied to the data.

Formula	Key to variables
	S_{obs} = the number of species in the sample.
$S_{Chao1} = S_{obs} + \frac{F_1^2}{F_2}$	F_1 = the number of observed species represented by a single individual (singletons).
$S_{Chao2} = S_{obs} + \frac{Q_1^2}{2Q_2}$	F_2 = the number of observed species represented by two individuals (doubletons).
	Q_1 = the number of species that occur in one sample only (unique species).
	Q_2 = the number of species that occur in two samples.
S_{ACE}	S_{rare} = the number of rare species (≤ 10 individuals).
$= S_{abund}$	S_{abund} = the number of abundant species (> 10 individuals).
$+ \left[\frac{S_{rare}}{C_{ACE}} - \frac{F_1}{C_{ACE}} \right] \gamma_{ACE}^2$	$C_{ACE} = 1 - F_1/N_{rare}$
	N_{rare} = the total number of individuals in rare species.
	γ_{ACE}^2 = estimates the coefficient of variance of the F_i 's.

Note: the idea behind these estimators is that if a community is being sampled, and species represented by only one individual (singletons) [F_1], unique species [Q_1] or rare species [S_{rare}] (according to the estimators Chao1, Chao2 and ACE, respectively) are still being discovered, there are likely still more singletons, unique species or rare species not found; as soon as all species have been recovered at least twice (doubletons) [F_2] (for Chao1) or species occur in two samples [Q_2] (for Chao2) or abundant species [S_{abund}] occur in the sample (for ACE), there are likely no more species to be found.

3.3. Results

3.3.1. Taxonomical composition of the entomofauna

The sampling campaign performed between September 2015 and September 2016 in the localities of San Lorenzo, Majes and La Molina yielded 301, 106 and 154 morphospecies, respectively, highlighting the orders Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera over the other orders (Annex 5).

3.3.2. Alpha diversity of the field sites

3.3.2.1. Structure of the community

The rank abundance curve of the morphospecies found at each locality is depicted in Figure 3.2. The corresponding curve for the field site in San Lorenzo has a less pronounced slope compared to the other two localities; this suggests that the entomofauna composition is more evenly distributed (in terms of abundance of species) than in La Molina and Majes. Examining the composition of the entomofauna, we found that 65% of the total abundance is formed by twelve morphospecies, with a non-identified species of Staphylinidae being the most abundant (12% of the total abundance).

The curve for Majes has the highest slope, suggesting that a small group of morphospecies dominates in the community over other taxa. Examining the composition of the entomofauna, we found that 65% of the total abundance is formed by a single species, *F. occidentalis*. The curve for La Molina has a similarly pronounced slope as that for Majes, with 65% of the total abundance being formed by four morphospecies, including *Macrosiphum euphorbiae* (Thomas) as the most abundant species (50% of the total abundance).

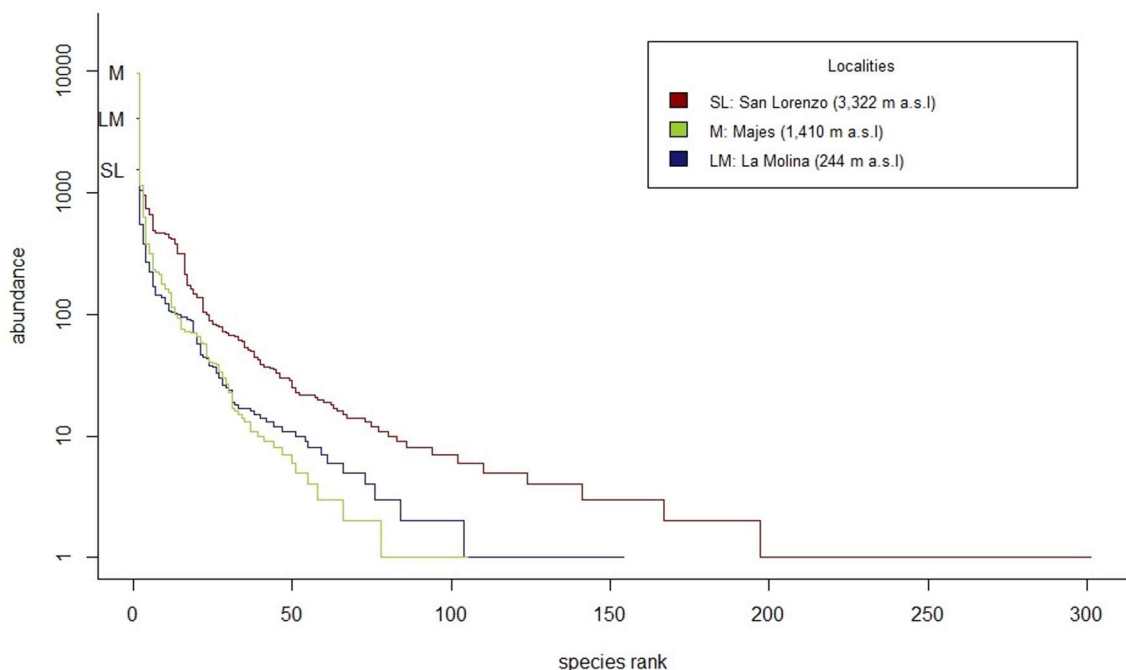


Figure 3.2. Rank abundance curve for the morphospecies found at the field sites in San Lorenzo, Majes and La Molina (log series distribution).

The Kruskal-Wallis test indicated significant differences between the compared field sites as to the diversity in terms of species equitability (Shannon index) and the dominance of certain taxa in the community (Simpson's dominance index) (Table 3.3).

The highest values of Shannon index were obtained in San Lorenzo, suggesting that this locality harboured a more evenly distributed entomofauna as compared to the other two field sites. Besides, the lowest value of Simpson's dominance index obtained at this locality indicates lower predominance of certain taxa in the community as compared to La Molina and Majes. Conversely, at the latter field site the lowest values of species equitability and the highest value of Simpson's dominance index were obtained. These results are in line with the abovementioned analyses.

3.3.2.2. Species richness

The Kruskal-Wallis test indicated significant differences between the compared field sites in regard to the diversity in terms of species richness (Margalef index) (Table 3.3).

The highest values of Margalef index were obtained in San Lorenzo, suggesting that this locality has a significantly larger number of species than the other two localities. The field site in Majes was characterized by the lowest values of species richness.

Table 3.3. Overall diversity indices (means \pm SE) calculated for the different localities.

Diversity index	Locality			Chi-Square	Degrees of freedom	p-value
	San Lorenzo*	Majes*	La Molina*			
Shannon	3.60 \pm 0.09 ^a	1.69 \pm 0.18 ^c	2.43 \pm 0.39 ^b	12.5	2	0.001
Simpson's dominance	0.05 \pm 0.003 ^a	0.44 \pm 0.07 ^c	0.27 \pm 0.11 ^b	11.8	2	0.003
Margalef	20.28 \pm 2.63 ^a	7.97 \pm 0.44 ^c	11.11 \pm 1.44 ^b	12.5	2	0.001

*Different letters (a, b, c) within a row indicate significant differences at $\alpha = 0.05$ (Kruskal-Wallis test).

3.3.2.3. Diversity within feeding guilds

The diversity of natural enemies (predators and parasitoids) and phytophagous insects was compared between localities (Table 3.4, Table 3.5).

The Kruskal-Wallis test indicated significant differences between field sites as to the diversity of phytophagous insects measured through the indices of species richness ($\chi^2_2 = 10.82$; $p < 0.01$) and of species evenness ($\chi^2_2 = 9.5$; $p < 0.01$). The field site in San Lorenzo had significantly higher values of Shannon and Margalef indices than those in Majes and La Molina.

Also for the natural enemies group significant differences between field sites were found in terms of species richness ($\chi^2_2 = 12.02$; $p < 0.01$) (Margalef index), with San Lorenzo being the locality with the highest value and Majes with the lowest. The three localities did not differ in terms of evenness ($\chi^2_2 = 4.02$; $p = 0.134$) (Shannon index).

Table 3.4. Diversity indices (means \pm SE) of phytophagous insects calculated for the different localities.

Diversity index	Locality			Chi-Square	Degrees of freedom	p-value
	San Lorenzo	Majes	La Molina			
Shannon	2.52 \pm 0.14 ^a	1.02 \pm 0.11 ^b	1.09 \pm 0.23 ^b	9.5	2	0.008
Simpson's dominance	0.11 \pm 0.01 ^b	0.6 \pm 0.05 ^a	0.59 \pm 0.10 ^a	9.4	2	0.009
Margalef	4.72 \pm 0.77 ^a	3.02 \pm 0.09 ^b	2.69 \pm 0.43 ^b	10.8	2	0.004

Different letters (a, b, c) within a row indicate significant differences at $\alpha = 0.05$ (Kruskal-Wallis test).

Table 3.5. Diversity indices (means \pm SE) of natural enemies calculated for the different localities.

Diversity index	Locality			Chi-Square	Degrees of freedom	p-value
	San Lorenzo	Majes	La Molina			
Shannon	2.35 \pm 0.08 ^a	2.18 \pm 0.11 ^a	2.22 \pm 0.17 ^a	4.0	2	0.134
Simpson's dominance	0.20 \pm 0.03 ^a	0.17 \pm 0.02 ^a	0.19 \pm 0.03 ^a	3.9	2	0.141
Margalef	10.37 \pm 1.60 ^a	3.85 \pm 0.56 ^c	5.52 \pm 0.98 ^b	12.0	2	0.002

Different letters (a, b, c) within a row indicate significant differences at $\alpha = 0.05$ (Kruskal-Wallis test).

The feeding guilds were also compared for each locality. Both for La Molina and for Majes the Mann-Whitney-Wilcoxon test indicated that the natural enemies group was more diverse (in terms of species evenness and species richness) than the phytophagous insect group ($\mathcal{W}_1 = 25$; $p < 0.01$). In San Lorenzo, the species richness of the natural enemy complex was significantly superior to that of the phytophagous insects ($\mathcal{W}_1 = 25$; $p < 0.01$) but there were no differences between these groups as to species equitability ($\mathcal{W}_1 = 4$; $p = 0.09$).

3.3.3. Estimation of the species richness

All estimators used suggest that the expected species richness at all field sites would be considerably superior to the observed richness (Table 3.6). According to these indices, San Lorenzo had higher expected species richness than the other field sites; using the Chao1 estimator, the expected number is up to 484 species (which is more than twice the number of species than in La Molina and more than three times than in Majes). Moreover, the low species richness in Majes is reflected in all estimators; based on the Chao2 estimator, the highest

expected number of morphospecies is at most 153, even if the sampling effort would be duplicated.

The sampling efforts were calculated based on the number of species observed and the expected number of species (Table 3.6). For La Molina between 68% and 71% of sampling effort was reached, very similar to San Lorenzo (between 62% and 71%). The Majes field site, however, had slightly higher values of sampling effort (between 70% and 76%).

Species accumulation curves were built based on the estimators Chao1, Chao2 and ACE, and on the number of species observed (Figure 3.3). The corresponding curves of the estimated species are quite far from the curve of the species observed for the three field sites. For La Molina, the curves tend to reach the asymptote while for Majes, the curves appear to be closer to the asymptote than for San Lorenzo.

Table 3.6. Mean estimated species richness with non-parametric indices and sampling effort for each field site at the localities under study.

Locality	S(obs)*	ACE Mean	Chao 1 Mean	Chao 2 Mean	Sampling effort
San Lorenzo	301	426.1	484.7	442.1	62-71%
Majes	106	140.1	141.0	152.4	70-76%
La Molina	154	225.9	219.0	216.3	68-71%

* S(obs): number of species sampled or observed.

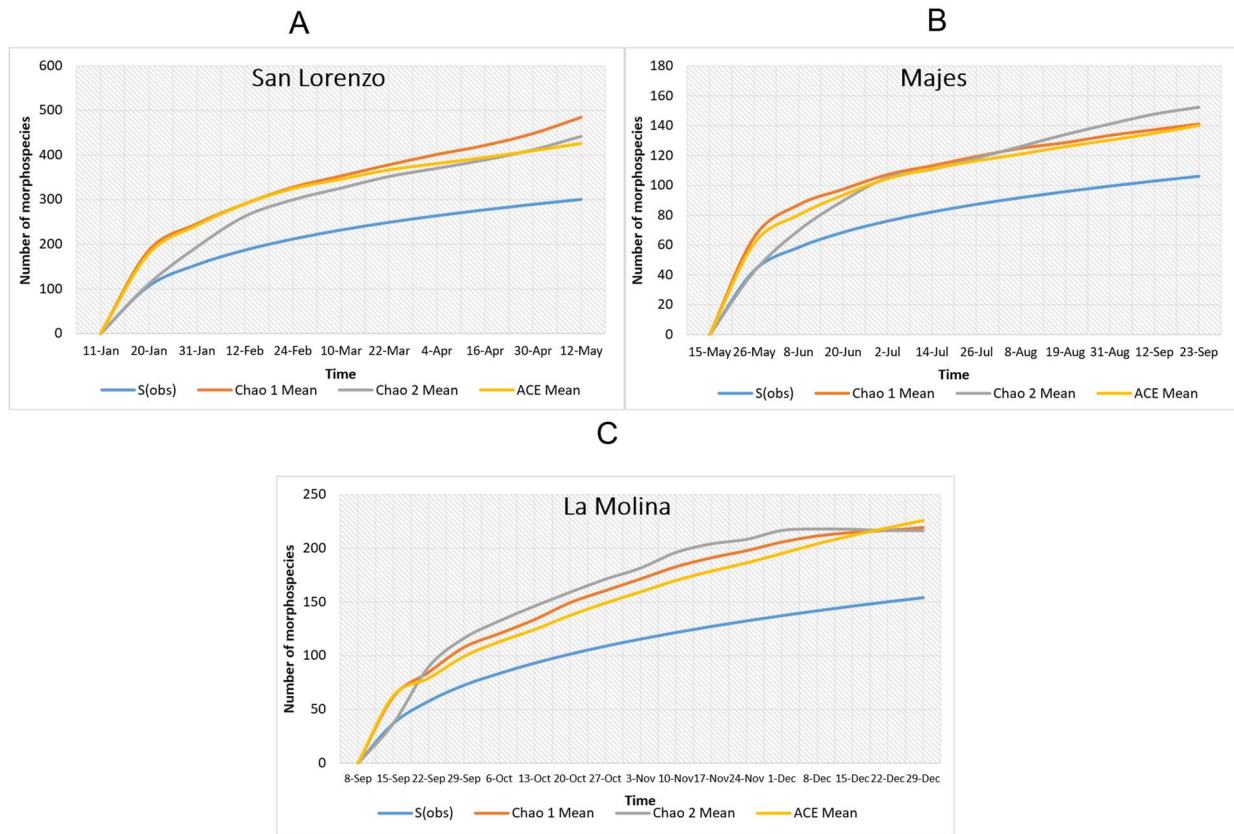


Figure 3.3. Accumulation curves of the species observed at the different field sites during the sampling campaign and the expected species accumulation curves according to the estimators Chao1, Chao2 and ACE: A. San Lorenzo, B. Majes, C. La Molina.

3.3.3. Beta diversity between localities

The Bray-Curtis index of dissimilarity indicated large differences between the entomofauna found at each locality (La Molina vs San Lorenzo = 0.95, La Molina vs Majes = 0.96, San Lorenzo vs Majes = 0.92).

The non-metric multidimensional scaling plot of the data obtained from the sampling at the three localities shows the ordination of the morphospecies according to the sites they belonged to (Figure 3.4). In this plot, species in common between two field sites are placed on the connecting dotted lines and species that were found in all three localities are inside the dotted triangle. The diagram indicates that the localities are very well separated from each other, implying that the entomofauna substantially differed between field sites. Thus, from the total of 544 morphotypes recorded in the sampling campaign, only a few inhabited two or three localities.

The morphospecies found at the San Lorenzo and Majes field sites were identified as *Macrosiphum* sp., *Myzus* sp. (Aphididae) and *Russelliana solanicola* Tuthill (Psyllidae); morphotypes sampled in La Molina and San Lorenzo were identified as *Epitrix* sp. (Chrysomelidae) and *Heterotrioza chenopodii* (Reuter) (Triozidae); and the species that inhabited La Molina and Majes were identified as *Blennidus peruvianus* (Dejean) (Carabidae), *Nysius simulans* Stål (Lygaeidae), *L. hyalinus* (Rhopalidae) and *Aphidius colemani* Viereck (Braconidae). The species collected at the three field sites were identified to be *F. occidentalis* (Thripidae), *Eurysacca melanocampta* (Meyrick) (Gelechiidae), and *L. huidobrensis* (Agromyzidae). Except for the carabid *B. peruvianus*, the aphidiine wasp *A. colemani* and the psyllid *R. solanicola*, all of the above have been reported as pests on quinoa (Rasmussen et al., 2003; Saravia et al., 2014; Yábar et al., 2002).

The PerMANOVA (run with 999 permutations) confirmed highly significant differences between localities ($F - model_{2,12} = 27.702$, $p = 0.001$). The pairwise comparison with permutation MANOVAs (with 999 permutations) indicated significant differences between the field sites of La Molina and Majes ($p = 0.007$), La Molina and San Lorenzo ($p = 0.004$) and between San Lorenzo and Majes ($p = 0.009$).

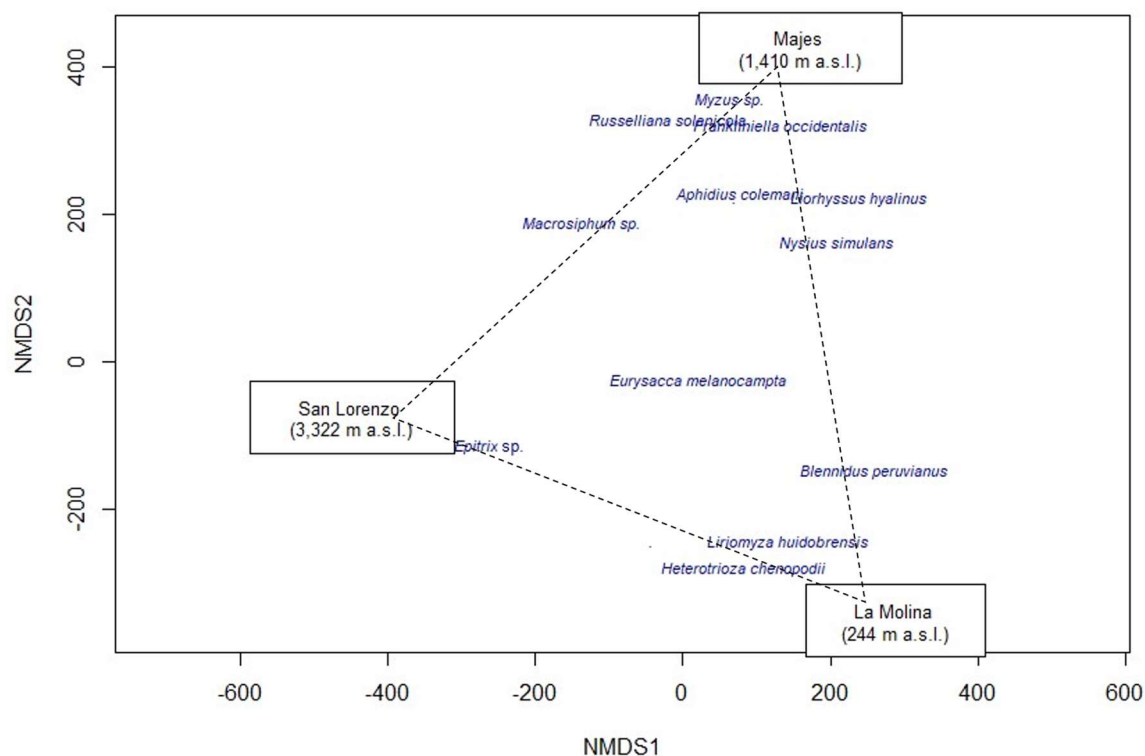


Figure 3.4 The NMDS plot showing the compositional distance between field sites of the three regions (San Lorenzo, Majes and La Molina) under study. Dotted lines represent the connection between sites with regard to the species in common.

The SIMPER analysis highlighted the most influential morphospecies that contributed to the large differences found between localities (Table 3.7).

Table 3.7. Contribution (%) of the most influential species to the differences between localities and their significance level.

Morphospecies	San Lorenzo – La Molina		San Lorenzo - Majes		La Molina - Majes	
	Contribution	p-value	Contribution	p-value	Contribution	p-value
<i>Frankliniella occidentalis</i>	0.93	0.999	36.28	0.001	42.92	0.001
<i>Macrosiphum euphorbiae</i>	20.28	0.001	-	-	18.31	0.001
<i>Myzus</i> sp.	1.72	0.999	3.41	0.062	5.29	0.002
Staphylinidae sp.3	7.62	0.001	5.95	0.001	-	-
Chloropidae sp.1	5.26	0.001	4.10	0.010	-	-
<i>Trimorus</i> sp.	5.10	0.001	3.93	0.005	-	-
Staphylinidae sp.1	3.84	0.001	2.98	0.003	-	-
<i>Phyllotreta</i> sp.	3.44	0.001	2.60	0.005	-	-
Psocoptera sp.1	-	-	2.55	0.006	2.98	0.001
<i>Chrysocharis</i> sp.	2.88	0.001	-	-	2.59	0.001
Formicidae sp.2	2.46	0.001	1.90	0.018	-	-
<i>Epitrix</i> sp.	2.38	0.001	1.88	0.010	0.03	0.998
Anthomyiidae sp.1	2.34	0.001	1.82	0.007	-	-
Sciaridae sp.1	2.20	0.001	1.71	0.003	-	-
<i>Macrosiphum</i> sp.	2.10	0.001	1.44	0.337	0.30	0.994
<i>Blennidus peruvianus</i>	1.95	0.001	0.14	0.997	1.60	0.002

3.4. Discussion

Hitherto, little attention has been paid to the study of the entomofauna associated with quinoa compared to other Andean crops such as corn or potato. Consequently, the literature provides limited information about the diversity of insects and the role they play in the agroecosystem of quinoa (Valoy et al., 2015). This type of information is even more scarce for the new production areas of quinoa at lower altitudes (Latorre, 2017). The present study aimed at elucidating the diversity and composition of the insect community in a traditional quinoa production zone, San Lorenzo (located in the Andes at 3,322 m a.s.l.), and two non-traditional zones of quinoa production, Majes (located in the ecoregion “Maritime Yunga” at 1,410 m a.s.l.) and La Molina (located in the coastal region at 244 m a.s.l.) from Peru.

The insects form a very diversified group whose population censuses in agroecosystems are constantly affected by agricultural practices; for this reason, it is unlikely that the asymptote in a species accumulation curve is reached in entomological surveys, even after an extensive

sampling campaign (Willott, 2001). In this context and considering that the sampling effort obtained (between 62% and 75%) for the three localities was quite similar, the results attained can be deemed reliable for comparing the insect communities.

Since the field sites under study correspond to quinoa monocultures, a low insect diversity is expected due to the less diverse vegetation. According to the literature, the Shannon diversity index usually falls between 1.5 and 3.5, and rarely surpasses 4, which only occurs when a huge number of species is found in the sample (Margalef, 1972). Taking this range as a reference, the Shannon index obtained in the locality of San Lorenzo can be considered high (mean 3.6) whereas for Majes and La Molina, low (mean 1.69) and intermediate (mean 2.43) values were obtained, respectively.

One of the factors that could be responsible for the high insect diversity found in San Lorenzo is its agroecological traits (Peacock & Worner, 2008). At this locality there are favourable conditions for a variety of insect species to prosper, i.e. the precipitation and loam soil type spark the development of a variety of volunteer plants (weeds and wild vegetation) that may serve as a refuge for insects moving to and from the quinoa plants (Wenninger & Inouye, 2008; Zhu et al., 2008; Bennett, 2010). Contrariwise, the desert environment in Majes, with only barren vegetation nearby, likely does not allow many insect populations to thrive through time, as it offers limited refuges, yielding low insect diversity. Finally, the environmental conditions at the field site in La Molina probably may have been the most favourable for insect diversity: an overall warmer climate with small differences between maximum and minimum temperatures, a variety of volunteer plants in the irrigation channels and field margins, and a clay loam soil type (Table 3.1) (Wallner, 1987; Sánchez & Vergara, 2003; Bennett, 2010; Mouhoubi et al., 2019). However, the agricultural practices at the latter field site likely had more influence on reducing the insect diversity than any adverse environmental variable (van Emden & Williams, 1974; McLaughlin & Mineau, 1995; Samways, 2005).

Another factor that may have affected the expected species richness and the abundance of insects, especially in Majes and La Molina, was the pest management scheme, more in particular the broad-spectrum insecticides used at these localities (Table 3.1). In Majes, the field was treated with four sprayings of pyrethroids during the first 60 days under a fixed schedule treatment, whereas in La Molina, two sprayings of organophosphates were done but based on the infestation level and damage of the pests observed in the field. This type of pesticides is usually preferred by local smallholders due to the cheaper price as compared with selective insecticides. However, broad spectrum compounds are considerably harmful to herbivores and

their natural enemies alike (Siegfried, 1993; Epstein et al., 2000), and may thus have been responsible for the lower insect diversity observed by pitfall trapping at these locations. Taking into account that the field site in Majes belonged to a private farm, the pest management performed there probably better reflects the way in which the smallholder farmers usually manage insect infestations. Local studies revealed that the most used active ingredient in the quinoa fields of Majes is cypermethrin while in Camacani (Puno) farmers generally use cypermethrin, methamidophos and lambda-cyhalothrin as active compounds to control pests, all of which are broad spectrum insecticides (Latorre, 2017).

The above mentioned situation suggests that there is a lack of knowledge among quinoa growers on the correct use of insecticides in regard to the mode of action of active ingredients, as classified by the Insecticide Resistance Action Committee (IRAC), in order to help prevent the development of insecticide resistance (Cisneros, 2012; IRAC, 2019). Also, many growers are unaware of the damage that this type of pesticides causes on the functional biodiversity of the agroecosystem.

Contrariwise to Majes and La Molina, in San Lorenzo selective insecticides were applied (*Bacillus thuringiensis* + emamectin benzoate) in only a single treatment and based on the infestation level of *E. melanocampta*. This may be another reason for the higher insect diversity observed in San Lorenzo as compared to the other localities (Navon, 2000; Ishaaya et al., 2002; Samways, 2005). Besides, the species richness of the entomophagous guild at this locality was also quite superior to that of the herbivores, which is in accord with previous reports pointing out that the agroecosystems of quinoa in the Andes can harbour a very diverse beneficial entomofauna (Quispe et al., 2014; Valoy et al., 2015).

Enhancing functional biodiversity is a key strategy to bring sustainability in plant production. For example, the natural enemy complex plays an important role in securing crop protection (Altieri, 1999). In the Andes, traditional farming is related to a sustainable agricultural system for a number of reasons (Latorre, 2017). Firstly, farmers only cultivate quinoa once a year because they depend on rain-fed irrigation, being forced to have a long fallow period which slows down the development of herbivorous insect populations throughout the year (García et al., 2015). Secondly, as many farmers from the highlands do not have sufficient economic resources, the use of chemical fertilizers and pesticides is sometimes limited (Camino et al., 1985; Camino & Johns, 1988; Halloy et al., 2005; Guerra García, 2006). Finally, farms in the Andes are generally small-scaled with crop parcels usually smaller than 1 ha. As a result, the valleys in the highlands consist of a variety of crops and non-crop plants (Orellano & Tillmann, 1984; National Research

Council, 1989; Jacobsen et al., 2003; Jacobsen, 2011; FAO, 2011). Thus, our results obtained at the field site in San Lorenzo seen in this context may also explain the greater insect diversity found at this locality compared to the other sites, as well as the superior diversity of the entomophagous guilds observed compared to that of the herbivores.

Taking this relatively high insect diversity in San Lorenzo into consideration, it may be more feasible to apply strategies of biological control at this traditional production locality than at the others, targeting the enhancement not only of beneficial species associated with the key pests but also other biological control agents that keep herbivores below pest threshold. Establishing integrated pest management programs may be a key point in conserving this local biodiversity.

The analysis of beta diversity unravelled that certain key pests of quinoa have a broad geographic distribution. The economically most relevant species inhabiting the three localities was *E. melanocampta*, which is considered a key pest of quinoa in the Andean region of Bolivia and Peru, the main quinoa production countries (Yábar et al., 2002; Rasmussen et al., 2003; Saravia et al., 2014). Its presence in the coastal zone of Peru (La Molina) suggests a broad adaptability and dispersion that farmers from the newly exploited areas should be aware of.

The leafminer fly *L. huidobrensis*, also found at the three locations, and the true bugs *N. simulans* and *L. hyalinus* found at the coastal level and in the “Maritime Yunga” ecoregion are considered to be secondary pests of quinoa (Cruces et al., 2016; Saravia et al., 2014). These also display a broad dispersion and adaptation to the different environments in which they were collected. Moreover, these species are polyphagous and are reported as pests of other crops which could serve as a source of infestation for quinoa (e.g. *L. huidobrensis* infests potato and tomato, *N. simulans* infests strawberry, *L. hyalinus* infests lettuce in seed production) (Alata-Cóndor, 1973; Korytkowski, 2014; Dughetti, 2015a). Thus, the disruption of existing top-down control mechanisms could turn into a problem for quinoa production in the new cultivation areas.

The results obtained in the present study may probably not reflect the true diversity of each locality, because crops are unstable systems, and the associated insect community may vary spatially and temporally. Therefore, further research is needed to better understand the insect diversity of studied localities, taking into account sampling more quinoa fields and natural vegetation nearby which is less disturbed by humans and more stable than crops along the time, and using other sampling techniques besides pitfall traps (e.g. pan traps). Nevertheless, this exploratory study provides relevant information about the expected insect diversity that can be found in quinoa fields which may assist in persuading quinoa growers (especially those from the Andean region) to enhance their agricultural practices in order to conserve the local insect

diversity and steward the ecosystem services provided by the natural enemy complex. Also, it can provide agroecology researchers with baseline data for future studies to unravel the diversity and role of the entomofauna of quinoa. Indeed, further research is also necessary to fully understand the consequences of the prevalent management tools in quinoa on the insect diversity and the pest – natural enemy interactions in different parts of the crop's production area of Peru as well as in other South American countries. In particular, this may be a key point for the success of organic quinoa production which is gaining international attention. Studies on the current use of chemicals in quinoa are also needed to better understand its impact on the diversity and abundance of the insects that visit the quinoa agroecosystems.

Chapter 4

Seasonal phenology of the major insect pests of quinoa (*Chenopodium quinoa* Willd.) and their natural enemies in a traditional zone and two new production zones of Peru

Redrafted after:

Cruces, L., de la Peña, E. and De Clercq, P. 2020. Seasonal phenology of the major insect pests of quinoa (*Chenopodium quinoa* Willd.) and their natural enemies in a traditional zone and two new production zones of Peru. *Agriculture*, 10(12), 644.

Author Contributions:

Conceptualization, L.C., E.d.I.P. and P.D.C.; methodology, L.C. and P.D.C.; formal analysis, L.C., E.d.I.P. and P.D.C.; investigation, L.C., E.d.I.P. and P.D.C.; data curation, L.C.; writing—original draft preparation, L.C.; writing—review and editing, L.C., E.d.I.P. and P.D.C.; supervision, E.d.I.P. and P.D.C.

4.1. Introduction

In the Andes of Peru, quinoa has mostly been cultivated as a staple crop by smallholders, with limited resources that do not allow them to use advanced agricultural technology. In this ecoregion, small-scale farming has largely been practiced, characterized by low inputs, the restricted use of machinery and rain-fed irrigation (Gómez-Pando et al., 2014; Gamboa et al., 2018). However, in the last years, as a consequence of the increasing demand for quinoa on the international markets and the resulting export boom and crop expansion, farmer associations have been created. In turn, this has led to improvements in crop management by the incorporation of agricultural machinery and technical assistance (Mercado & Ubillus, 2017). The production of this Andean grain in the highlands is mostly organic, with a relatively low yield level that is partially compensated by the higher market price as compared with conventional quinoa (Montero & Armando, 2017; Bedoya-Perales et al., 2018a).

This revalorization of quinoa motivated many farmers in the Andes to shift from staple crops (such as potato, corn and legumes) to quinoa but also gained attention of growers from regions at lower altitudes (i.e., from the “Maritime Yunga” to the coastal areas) (Gómez-Pando et al., 2014; Bedoya-Perales et al., 2018a, 2018b). In these newly exploited areas, small-, medium- and large-scale cultivation is practiced, characterized by the implementation of relatively advanced farming techniques including technified irrigation (especially in areas belonging to local irrigation projects such as “Majes-Siguas” and “Olmos” in the Arequipa and Lambayeque departments, respectively) and the use of machinery, pesticides, fertilizers and, in some cases, modern equipment for spraying (Cruces et al., 2016; Latorre, 2017; Montero & Armando, 2017). Therefore, the production of quinoa in these areas is mainly conventional, with higher yield levels than in the highlands (Gómez-Pando et al., 2014; Montero & Armando, 2017; Bedoya-Perales et al., 2018a).

A relatively long list of phytophagous insects has been reported to infest quinoa in the Andean areas (Cruces et al., 2016; Cruces et al., 2020a). However, only the quinoa moths *Eurysacca melanocampta* (Meyrick) and *Eurysacca quinoae* Povolný (Lepidoptera: Gelechiidae) are considered of major importance, while other herbivorous species, including thrips and aphids, are generally considered of minor relevance (Saravia et al., 2014; Valoy et al., 2015). For the non-traditional areas of quinoa production, pest communities infesting the crop also include *E. melanocampta*, as well as polyphagous insects such as the aphid *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae), the thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), the leafminer fly *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) and the hemipteran pests *Nysius simulans* Stål (Hemiptera: Lygaeidae) and *Liorhysus hyalinus*

(Fabricius) (Hemiptera: Rhopalidae) (Chapter 3). Knowledge about the economic impact of the latter pests on quinoa production in the newly exploited areas is, however, still scarce.

In this context, the present study aimed to explore the seasonal occurrence of the relevant insect pests on quinoa in two new production zones as compared to a traditional production area, by analysing their incidence in the crop, as a function of the presence of their natural enemies, environmental factors and the farming practices specific to each region. The findings of this study should be of interest for local quinoa growers for improving their pest management practices and also for other farmers who intend to explore new areas for quinoa production in Peru and other countries that share similar pest complexes.

4.2. Materials and Methods

4.2.1. Field sites

The study was carried out in Lima and Arequipa (two non-traditional quinoa production regions in Peru) and Junín (the traditional quinoa production region in Peru) which served as a reference for comparisons and analysis. The characteristics of each field site and growing specifications were described in chapter 3 (Table 3.1, Annex 2,3). Meteorological data (temperature and relative humidity) during the growing season of the studied zones is presented in Figure 4.1.

4.2.2. Sampling procedure

The sampling campaign was performed considering the planting season for each location, and samples were taken evenly throughout the crop phenology, from two weeks after germination to one week before harvest. In La Molina, 15 samplings were performed from 22/09/2015 to 29/12/2015; in Majes, 10 samplings were performed from 26/05/2016 to 12/09/2016; and in San Lorenzo, 9 samplings were performed from 31/01/2016 to 12/05/2016. The lower number of samplings executed in Majes and San Lorenzo as compared to La Molina was due to the lesser accessibility of the first two sites.

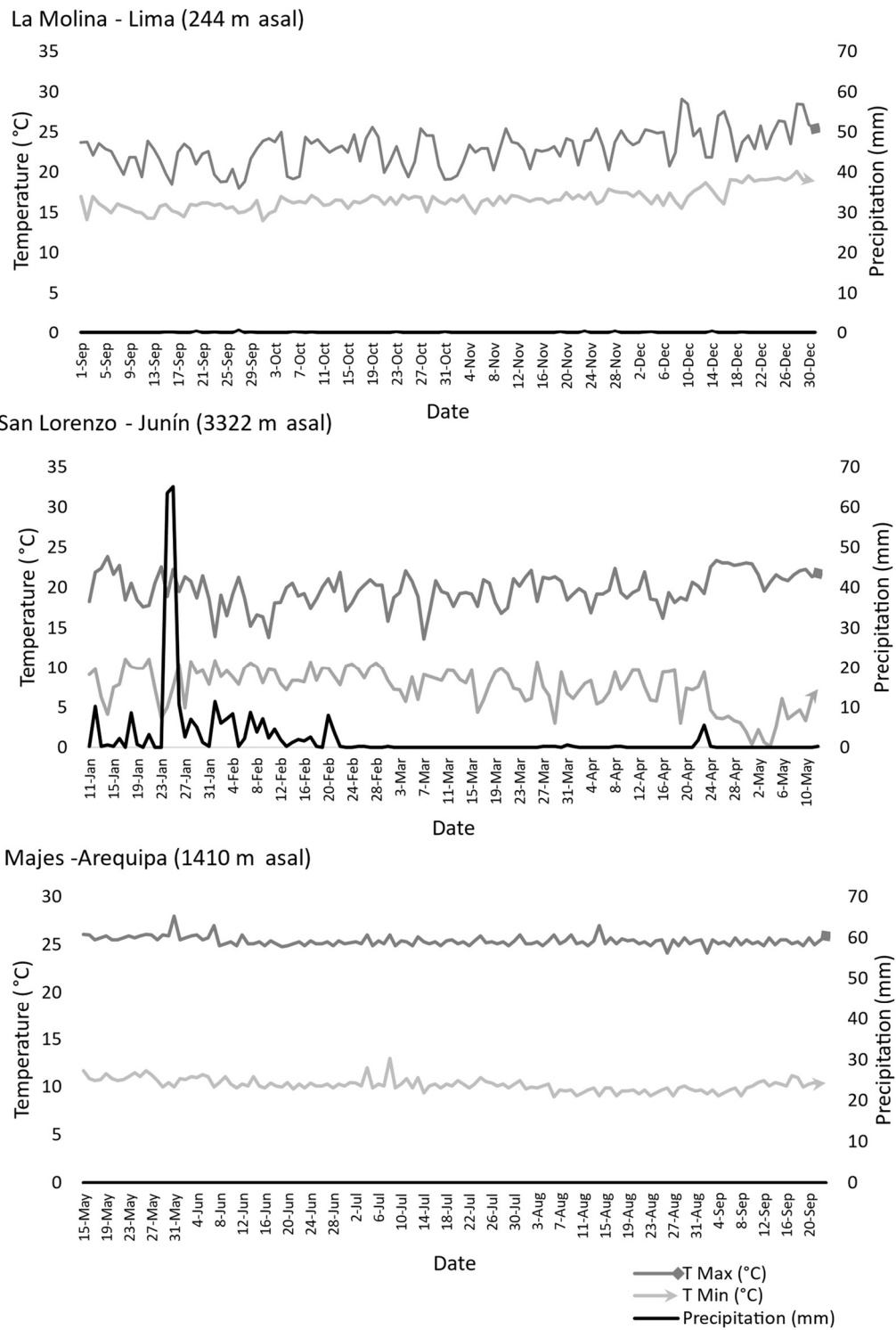


Figure 4.1. Fluctuation of the daily maximum and minimum temperatures and daily precipitation in Lima - La Molina (from 01/09/2015 to 30/12/2015), Junín - San Lorenzo (from 11/01/2016 to 12/05/2016) and Arequipa - Majes (from 15/05/2016 to 29/09/2016).

At each location, the field was divided into 5 sectors (considering the slope of the field and the irrigation blocks); in each sector, 5 quinoa plants, at least 20 m apart, were sampled (Figure 4.2). Each sampled plant was cut at its base and placed into a container with water, alcohol and some drops of liquid detergent. After taking five plants per sector, they were carefully chopped into small pieces, and the whole sample (including the liquid content) was transferred to a labelled, airtight container to be transported to the laboratory for further processing. Plants from borders were always avoided for sampling. During collection, care was taken to minimize the disturbance of any insects present on the plant.

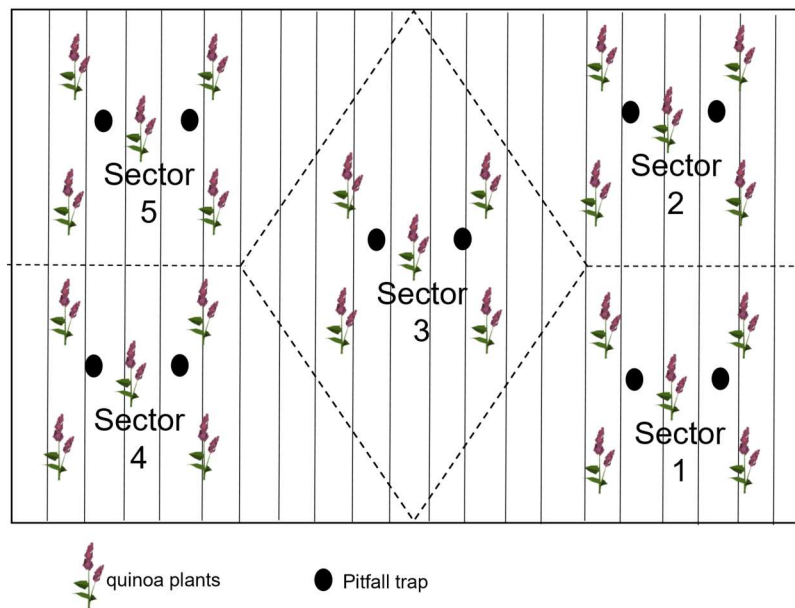


Figure 4.2. Sectorization and sampling scheme applied to the monitored fields. Transversal lines represent the direction of the furrows.

To complement the analysis, the epigeous insects were examined throughout the crop phenology with ten pitfall traps (transparent, \approx 10 cm, 10% ethylene glycol, water and detergent) and 2 traps per sector (Figure 4.2), which were left during the whole crop phenology (from one week after germination to one week before harvest). The pitfall trap content was periodically collected on the same day when the quinoa plants were sampled (Chapter 3).

4.2.3. Sample processing and identification

All samples were processed at the laboratories of the Museum of Entomology “Klaus Raven Büller” of the National Agrarian University La Molina, in Lima, Peru, where the collected specimens were deposited.

The recipients containing the sampled plants and pitfall trap samples were poured onto a 1 mm mesh sieve and carefully washed with water, removing larger materials, except for the leaves with mines; these were later examined under a binocular stereoscope (Carl Zeiss, Stemi 508 LAB, Zeiss, Jena, Germany) to remove the leafminer larvae and/or their parasitoids. The remaining samples (i.e., the collected insect specimens) were transferred to labelled glass vials containing 75% v/v ethanol for conservation and further processing (i.e., identification).

The specimens were sorted on the basis of morphological characteristics as morphospecies. For the paurometabolous insects, adults and nymphs were taken into account, but for holometabolous insects, only the harmful stages (larvae and/or adults) were considered in the study. For the aphids, mummified specimens were also considered, to calculate the parasitism level based on the number of parasitized aphids and the total number of aphids collected. For the leafminers, the parasitism level was calculated based on the number of parasitoids and leafminer larvae extracted from the mines.

When feasible, the most relevant morphospecies (taking into account abundance and functional behaviour) were identified at the genus and species levels, with the help of taxonomic keys and morphological descriptions provided in the literature as follows: for Aphididae spp. (Blackman & Eastop, 2000, 2006), Aphidiinae spp. (Sary, 1973; Carver & Franzmann, 2001; Kavallieratos et al., 2010, 2013), *Allograpta exotica* (Wiedemann) (Castro & Araya, 2012), *Blennidus peruvianus* (Dejean) (Straneo, 1986; Moret, 1995, 2003; Bousquet, 2010), *Diabrotica sicuanica* Bechyne (Krysan et al., 1984), *Epitrix* spp. (Biondi & D'Alessandro, 2012), *Eulophidae* genera (Reina & La Salle, 2003), *E. Melanocampta* (Povolný, 1986), *Geocoris* spp. (Henry et al., 2015), *Halticoptera* sp. (Bouček & Rasplus, 1991), *Heterotrioza chenopodii* (Reuter) (Horton et al., 2018), *L. hyalinus* (Göllner-Scheiding, 1976; Cornelis et al., 2012), *L. huidobrensis* (Spencer, 1973; Korytkowski, 2014), *Nabis capsiformis* Germar (Cornelis & Coscarón, 2013), *N. simulans* (Pall et al., 2016) and *Russelliana solanicola* Tuthill (Hodkinson & White, 1979; Burckhardt, 1987a).

Molecular tools were applied for identifying and/or confirming the species *Lysiphlebus testaceipes* (Cresson), *Aphidius matricariae* Haliday, *Aphidius colemani* Viereck, *Aphidius rosae* Haliday, *Aphidius avenae* Haliday, *Aphidius ervi* Haliday, *F. occidentalis*, *L. huidobrensis*, *L. hyalinus*, *M. euphorbiae* and *Rhopalosiphum rufiabdominale* (Sasaki). DNA extraction and PCR procedures were performed in the Laboratory of Agrozoology, Department of Plants and Crops at Ghent University, Belgium, following specific protocols provided in the literature (Shufran & Puterka, 2011; Derocles et al., 2012; Nakamura et al., 2013; Harbhajan & Kaur, 2017; T. Ding et al., 2018). Specimens of *Epitrix* sp., *Macrosiphum* sp., *Myzus* sp., *Therioaphis* sp., *Geocoris* sp.,

Chrysocharis sp., *Halticoptera* sp., *Diglyphus* sp. And *Closterocerus* sp. Could not reliably be identified at the species level, either morphologically (since this is only confirmed by a specialist of the corresponding taxa) or based on molecular methods.

Expert taxonomists assisted by identifying and/or confirming certain species: *H. chenopodii* and *R. solanicola* were identified by Daniel Burckhardt from the Naturhistorisches Museum of Switzerland; the dolichopodids were identified by Daniel Bickel from the Australian Museum; *Astylus subannulatus* Pic was identified by Robert Constantin from the Entomological Society of France; *N. simulans* was identified by Pablo Dellapé from the Museo de La Plata in Argentina.

4.2.4. Data analysis

For the most relevant species (major pests and their natural enemies), curves of seasonal occurrence were built to analyse the pest–natural enemy interactions, which were interpreted in the context of each scenario (i.e., the environmental factors and the agricultural practices at each field site).

The statistical analyses were performed using the R software, version 3.4.2 (R Core Team, 2020) (packages: *vegan*, *agricolae*, and *MASS*) (Oksanen et al., 2020; De Mendiburu, 2020; Ripley et al., 2020).

For the population comparisons, a one-way ANOVA was applied to the data after having tested the normality and homoscedasticity through Shapiro–Wilk and Bartlett tests, respectively. When the data did not meet the assumption of the homogeneity of variances, the Box–Cox transformation method was used to stabilize the variances. When the ANOVA was significant, Tukey’s honestly significant difference test was used to compare the groups. All the tests were analysed at the significance level of $\alpha = 0.05$.

4.3. Results

4.3.1. Abundance and diversity of phytophagous insects

The plant samplings throughout the crop phenology at the field site in La Molina yielded 24 morphospecies of phytophagous species, among which *M. euphorbiae*, *E. melanocampta*, *F. occidentalis*, *L. huidobrensis* and *H. chenopodii* encompassed 99.1% of the total abundance of herbivorous insects. At the field site in Majes, 12 morphospecies of phytophagous insects were found, including *F. occidentalis*, *Myzus* sp. And *Macrosiphum* sp., encompassing 99.2% of the total abundance of herbivorous insects. The hemipteran pests *L. hyalinus* and *N. simulans*, which were recently reported to be causing severe damage in newly exploited areas for quinoa

production, were found at low densities at these two localities (Cruces et al., 2016; Latorre, 2017). Finally, in San Lorenzo, 16 morphospecies of phytophagous insects were found, with *F. occidentalis*, *E. melanocampta*, *Myzus* sp., *Macrosiphum* sp. And *H. chenopodii* accounting for up to 97.3% of the total abundance of herbivores. At this locality, *A. subannulatus*, *D. sicuanica* and *Epitrix* sp., which are mentioned in the literature as minor pests of quinoa (Krysan et al., 1984; Saravia et al., 2014; Cruces et al., 2016), were collected in very small numbers.

Rank–abundance curves of phytophagous insects were built as a function of their abundance in the samplings at each field site (Figure 4.3). Comparatively, the curve for the San Lorenzo field site (SL) has a less pronounced slope than the curves for the other sites. This suggests that the phytophagous species are more evenly distributed at this locality or there was a lower dominance of the most abundant pests as compared to at the La Molina and Majes field sites, which were characterized by a higher dominance of certain taxa.

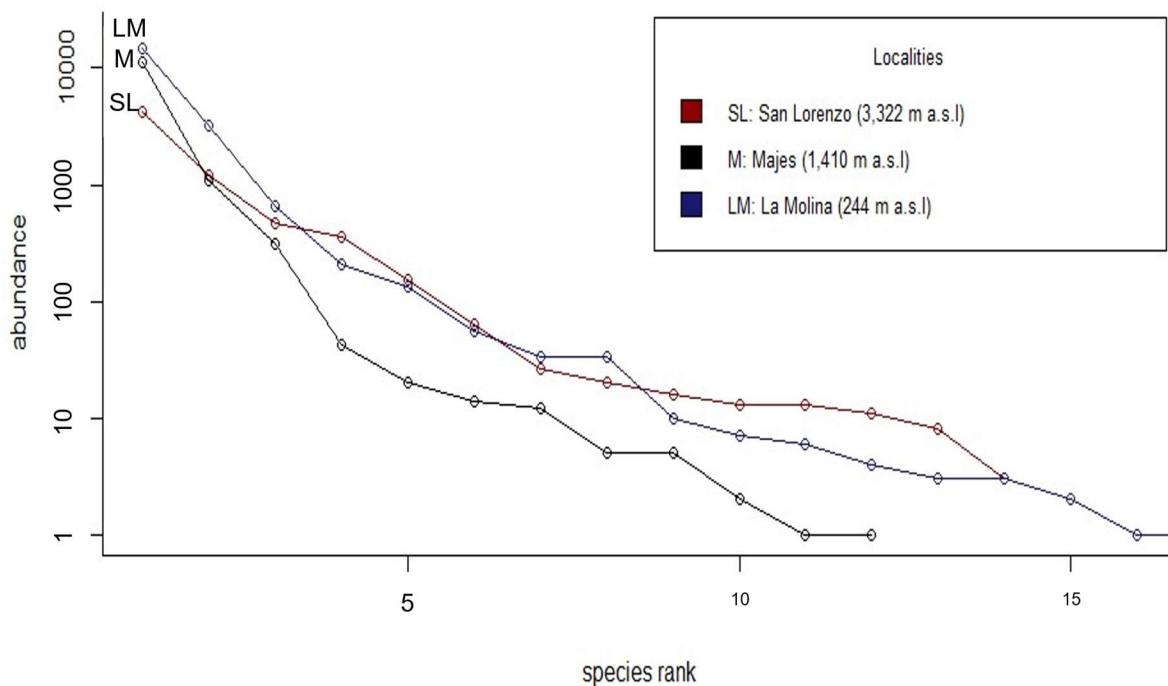


Figure 4.3. Rank–abundance curve of the phytophagous insects that infested the quinoa crop in San Lorenzo, Majes and La Molina (log series distribution).

4.3.2. Phenology of phytophagous insects of economic importance

4.3.2.1. Quinoa moth

At the field site in La Molina, the seasonal occurrence curve of *E. melanocampta* (Figure 4.4), based on the number of larvae per plant, had two peaks throughout the crop phenology. The first peak occurred on 03/11/2015, with an average of 7.9 individuals per plant; this was

controlled with the insecticide treatment dimethoate + methomyl (Table 3.1), from which the pest later resurged. The second peak occurred on 8/12/2015, with up to 65.6 specimens per plant on average; this infestation was managed with emamectin benzoate + methomyl, leading to a marked suppression of this pest. The first spraying with *Bacillus thuringiensis* var. *kurstaki* performed on 27/10/2015 against a low population of this moth had little effect.

Caterpillars of this species were scarcely observed in Majes, likely due to the constant treatments with broad-spectrum insecticides during the first 60 days of the cropping season.

At the field site in the traditional quinoa production locality, San Lorenzo, the occurrence of *E. melanocampta* larvae had its maximum number on 04/04/2016 (Figure 4.6). The caterpillars started to infest the plants 44 days after sowing (24/02/2016) and progressively increased in number up to 15.1 larvae per plant, on average. At this point, they were controlled with emamectin benzoate + *B. thuringiensis* var. *kurstaki*, which efficiently reduced the larval incidence thereafter.

With regard to the environmental variables (Figure 4.1), in San Lorenzo, the rain had a notorious effect on the establishment of this moth in the field, since the infestation began only after the raining period had finished (at the end of February). Minimum temperatures that mostly ranged between 0 and 10 °C likely also had an effect on the moth, slowing down its incidence. Contrarily, precipitation at the locality of La Molina was scarce, and the temperature was quite stable throughout the cropping season, with small differences between the maximum and minimum; thus, the interaction between these environmental factors and *E. melanocampta* incidence was not evident. Additionally, no specialized natural enemies of *E. melanocampta*, such as parasitoids, were observed during the sampling campaign, either at La Molina or at San Lorenzo.

The mean density of *E. melanocampta* larvae sampled on the plants at La Molina and San Lorenzo over the total sampling period was compared. After applying the Box–Cox transformation method ($\gamma = -0.5$) to the data, the ANOVA indicated that the overall larval density was significantly higher in La Molina than in San Lorenzo ($F_{1,8} = 31.46$, $p < 0.001$).

4.3.2.2. Aphid–natural enemy complex

The infestation by aphids at the field sites was related to more than one species: At the locality of La Molina, a high incidence of *M. euphorbiae* (99.8%) and scarcely any *R. rufiabdominale* (0.2%) were found; in Majes, *Myzus* sp. (77.3%) and *Macrosiphum* sp. (22.7%) were observed;

and in San Lorenzo, the aphid complex consisted of *Myzus* sp. (55.7%), *Macrosiphum* sp. (42.8%) and *Therioaphis* sp. (1.5%).

The seasonal occurrence curve of *M. euphorbiae*, based on the number of aphids per plant, had two peaks in La Molina (Figure 4.4). The first occurred on 03/11/2015, with the highest recorded population (162.3 individuals per plant on average), promoting the development of sooty mould on the leaves as a consequence of their honeydew secretion; this infestation was controlled efficiently with methomyl + dimethoate. The second peak occurred on 24/11/2015 (with 44.8 specimens per plant on average), but at this point, no insecticide was used, so the corresponding reduction of the aphid population in the following days may, in part, be explained by the action of the natural enemies, especially chrysopid larvae, the population of which increased in this period.

According to seasonal changes in the aphid abundance in La Molina, a temporal succession in the numerical response of the aphidophagous guilds was observed (Figure 4.4). Larvae of the predatory syrphid *A. exotica* first appeared, with peak numbers in the early developmental period of the aphid population, followed by aphidiine wasps but with a maximum parasitism level of only 2.5%; at the later phases of the crop, chrysopid larvae were found again. Wasps of the Aphidiinae complex collected in the pitfall traps consisted of *L. testaceipes* (Cresson), *A. matricariae* and *A. colemani*.

In Majes, the incidence of Aphididae was very low during the first 60 days after sowing (15/09/2016–14/07/2016), probably due to the intensive insecticide treatments applied in the early stages of the crop. From then onwards, the infestation continuously grew, reaching up to 22.5 individuals per plant on average (on 31/08/2016), followed by a decrease that may, in part, be explained by the action of predators such as chrysopid and coccinellid larvae, and parasitism by Aphidiinae wasps (Figure 4.5). When examining the specimens belonging to this group collected in the pitfall traps at Majes, the complex was formed by *A. colemani*, *A. ervi*, *A. avenae* and *A. rosae*.

Contrarily to the field site in La Molina, syrphids were absent in Majes, and the most abundant aphidophagous group was the Aphidiinae wasp complex. These appeared in the early stages of the crop, but their establishment became more significant after the period of insecticide treatments, during the grain formation and maturation, with a maximum parasitism level of 13.5%. Coccinellid and chrysopid larvae appeared in small numbers, also at the end of the crop phenology (Figure 4.5).

At the field site in San Lorenzo, the incidence of the Aphididae was considerably lower than in La Molina, amounting to only 7.1 specimens per plant, on average (Figure 4.6). Given this low infestation, no pesticide treatment was applied against the aphids and the spraying with emamectin benzoate + *B. thuringiensis* targeted against *E. melanocampta* larvae had no visible effects on the Aphididae. Based on the number of aphid specimens sampled per plant, there was a quite stable population density until 84 days after sowing (04/04/2016), followed by a slight increase.

When juxtaposing the environmental variables (Figure 4.1) and the aphid occurrence, only in San Lorenzo can a certain interaction be observed: for example, the aphid establishment at the beginning of the crop phenology only prospered when the rains subsided; also, the large differences between the maximum and minimum temperatures and chilling conditions in the period from 28/04/2016 to 04/05/2016 coincided with a decrease in the aphid population. These factors may also have affected the abundance of the natural enemies since only a single larva of Syrphidae and six larvae of Chrysopidae were collected throughout the crop phenology, and the maximum parasitism level reached no more than 7.2% during the cropping season (Figure 4.6). In this locality, *A. colemani* and *Aphidius* sp. were recorded in the pitfall traps.

The mean overall densities of Aphididae at the three localities were compared. After applying the Box–Cox transformation method ($\gamma = 0.1$) to the data, the ANOVA indicated that there were highly significant differences between the localities ($F_{2,12} = 146.4$, $p < 0.001$). Tukey's HSD test indicated that the aphid density in La Molina was significantly higher than in San Lorenzo ($p < 0.001$) and Majes ($p < 0.001$), the latter locality having a significantly higher aphid incidence than San Lorenzo ($p = 0.033$).

4.3.2.3. Leafminer flies and natural enemy complex

Adults and larvae of *L. huidobrensis* were found in considerable abundance only in La Molina, and therefore, the seasonal occurrence of this species was analysed in detail only for this locality. Since the adults of leafminer flies are very active and easily disturbed, they could not be efficiently sampled by way of the plant sampling, and therefore, the collected adult data were excluded from analysis.

The seasonal occurrence of *L. huidobrensis* had a maximum number of 3.3 larvae per plant (Figure 4.4). This infestation level was reduced by the treatment with methomyl + dimethoate targeted against aphids on 03/11/2015. Later, the parasitoid complex, formed mainly by eulophids and pteromalids (Mujica & Kroschel, 2011), had an important role in decreasing the leafminer population, with parasitism reaching up to 100% (Figure 4.4).

When examining the specimens collected in the pitfall trap sampling, the following leafminer fly parasitoids were recorded: two species of Pteromalidae (*Halticoptera* sp.1 and *Halticoptera* sp.2) and seven of Eulophidae (*Chrysocharis* sp.1, *Chrysocharis* sp.2, *Diglyphus* sp.1, *Closterocerus* sp.1, *Cirrospilus* sp.1 and two non-identified taxa). From this complex, *Halticoptera* sp.1 and *Chrysocharis* sp.2 were present in markedly larger numbers than the others.

4.3.2.4. Hemipteran pests

The rhopalid *L. hyalinus* was only collected in the non-traditional quinoa production localities La Molina and Majes, but in small numbers. In the first locality, only six specimens of this species were found, in the last plant sampling. In Majes, the population size was greater and focused in the grain filling stage (Figure 4.5), although the mean density of this bug on the plants never surpassed 0.68 specimens per plant, with a large standard deviation, suggesting that the spatial distribution of this species in the crop is not uniform but clumped.

The lygaeid *N. simulans* was also collected only at the localities of La Molina and Majes. Since this species has a primarily soil-surface-dwelling behaviour, the seasonal occurrence was analysed, contrasting the population found on the plants with the specimens collected in the pitfall traps.

In La Molina, the population of *N. simulans* at ground level was characterized by a considerable increase from the grain filling stage onwards, and the insect started to inhabit the plants around the physiological maturation stage (Figure 4.4). The field eventually had a strong outbreak of this bug from the harvest cut to the day of threshing; unfortunately, the population size at that time could not be recorded because the last sampling was performed one week before cutting. Since the cut plants were lying on the soil surface during 10 days for drying, this greatly favoured the infestation of quinoa by *N. simulans*.

In Majes, the occurrence of *N. simulans* at the soil level remained low until the grain filling stage, when the bugs also started to infest the plant; from then onwards, the population constantly increased, reaching up to 4.9 individuals per pitfall trap, on average, in the last sampling. On the plant, the population size remained small, reaching only 0.32 individuals per plant, on average, in the last sampling (Figure 4.5).

4.3.2.5. Western flower thrips

The seasonal occurrence curve of *F. occidentalis* in La Molina was characterized by two peaks (Figure 4.4). The first occurred on 03/11/2015, reaching only 4.5 individuals per plant on average, but the infestation was likely reduced by the insecticide treatment (methomyl +

dimethoate) targeted against the aphids and *E. melanocampta*. The second peak occurred on 08/12/2015, reaching 5.2 individuals per plant on average, whereafter the thrips incidence was likely reduced by the insecticide treatment (methomyl + emamectin benzoate) applied to control *E. melanocampta*. These pesticide sprayings may have obscured the interactions between the thrips and certain generalist natural enemies such as *N. capsiformis* and chrysopids found in the samplings.

The seasonal occurrence curve of *F. occidentalis* in Majes had an exponential shape, reaching up to 198 thrips per plant on average, in the last sampling. The population at the early stage of the crop phenology was small, probably due to the intensive use of insecticide during this phase. Thereafter, the infestation had a continuous increase, suggesting that there were few restrictive factors for the population growth during the monitored period; thus, natural enemies such as chrysopid larvae appeared to have had little effect on the thrip infestation (Figure 4.5).

The seasonal occurrence of *F. occidentalis* in San Lorenzo had a maximum number of up to 41.7 thrips per plant on average (Figure 4.6). It is likely that the minimum temperatures between 28/04/2016 and 04/05/2016, with values going down to 0 °C, had a detrimental effect on this pest (Figure 4.1).

The mean densities of the *F. occidentalis* per plant sampling at the three field sites were compared. After applying the Box–Cox transformation method ($\gamma = 0.1$) to the data, the ANOVA indicated that there were highly significant differences between the localities ($F_{2,12} = 226.8$, $p < 0.001$). Tukey's HSD test showed that the thrips density in Majes was overall significantly higher than in La Molina ($p < 0.001$) and San Lorenzo ($p < 0.001$); the density at the latter site was significantly greater than at La Molina ($p < 0.001$).

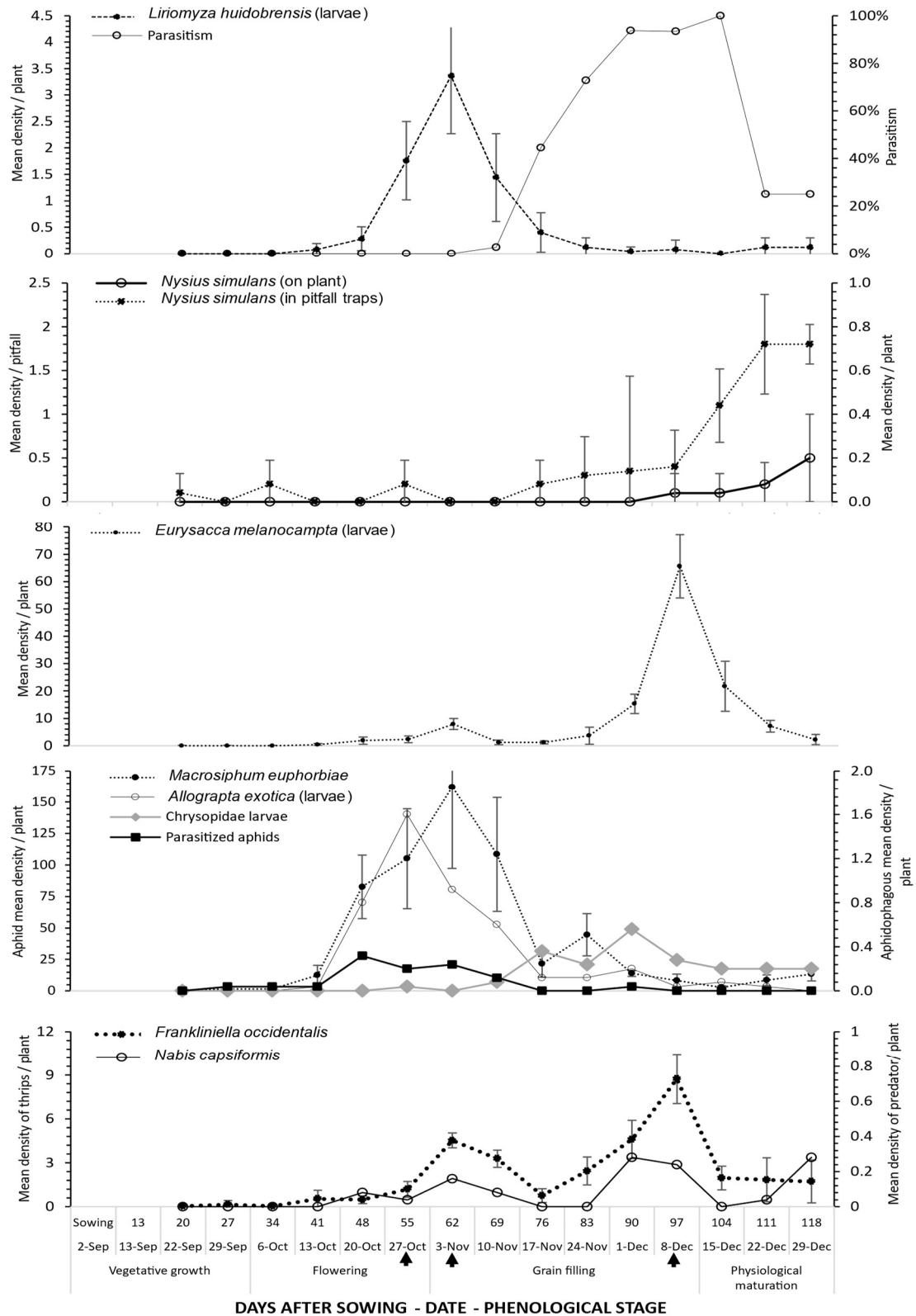


Figure 4.4. Seasonal incidence of the main insect pests (mean number per plant or pitfall trap \pm SD) and their associated natural enemies (mean number per plant or percent parasitism) sampled on quinoa at the field sites in La Molina, Lima (from 22/09/2015 to 29/12/2015). Arrows on the time axis indicate the timing of the insecticide applications.

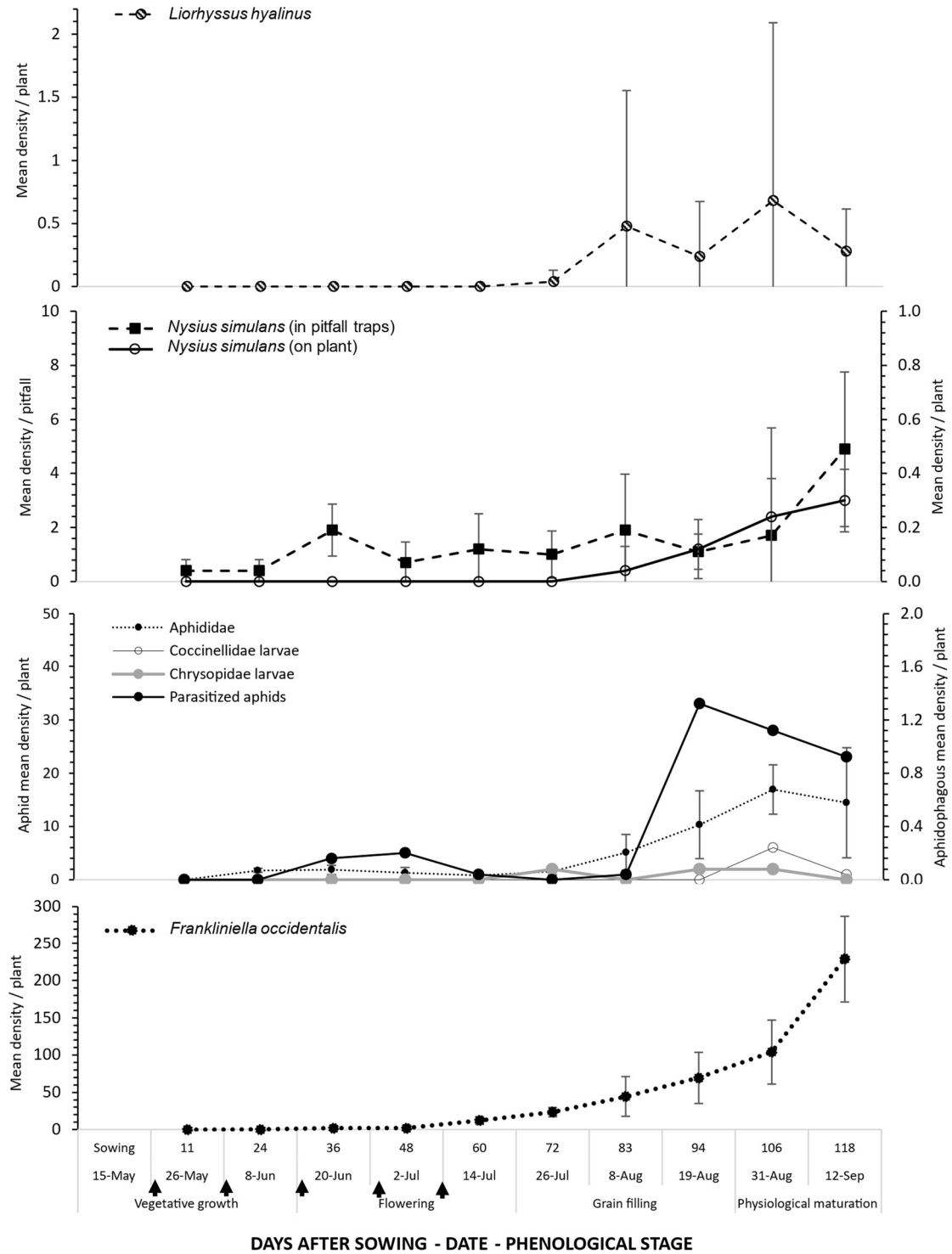


Figure 4.5. Seasonal incidence of the main insect pests (mean number per plant or pitfall trap \pm SD) and their associated natural enemies (mean number per plant) sampled on quinoa at the field sites in Majes, Arequipa (from 15/05/2016 to 12/09/2016). Arrows on the time axis indicate the timing of the insecticide applications.

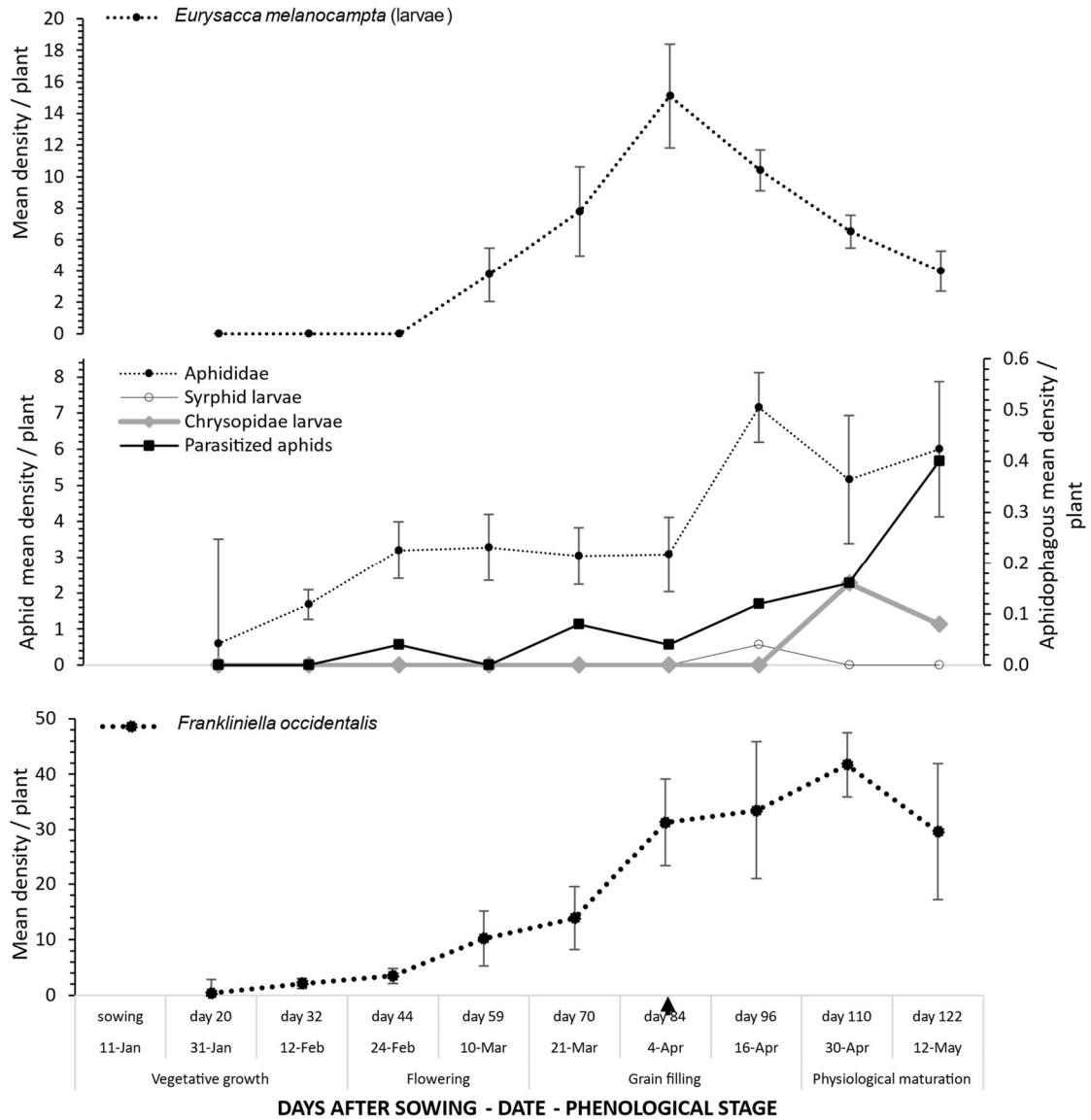


Figure 4.6. Seasonal incidence of the main insect pests (mean number per plant \pm SD) and their associated natural enemies (mean number per plant) sampled on quinoa at the field sites in San Lorenzo, Junín (from 31/01/2016 to 12/05/2016). Arrows on the time axis indicate the timing of the insecticide applications.

4.4. Discussion

The survey at the field in San Lorenzo confirmed the relevance of *E. melanocampta* for quinoa in the Andes of Peru, which is deemed, in the literature, to be the crop's key pest (Yábar et al., 2002; Rasmussen et al., 2003; Saravia et al., 2014). Likewise, the findings in La Molina shed light on the importance of this moth at the coastal level, a newly exploited region for quinoa production (Cruces et al., 2016), and revealed that polyphagous insects such as *M. euphorbiae* and *L. huidobrensis* may infest quinoa plants in high densities. Nonetheless, similar observations could not be made in Majes, where pest insects were scarcely collected in the early stages of the crop, likely due to the pest management scheme (Table 3.1), and only the population of the cosmopolitan pest *F. occidentalis* prospered in high densities when the insecticide sprayings stopped.

In the highlands of Peru, most of the cultivated quinoa is rain-fed irrigated. For this reason, farmers only cultivate the crop during the raining season, being forced to have a fallow period (Gómez-Pando et al., 2014). In this context, *E. melanocampta* may have two generations in the Andean region (Quispe et al., 2014); the first occurs between November and December in early sowings, and the second is between March and April for late sowings, the latter coinciding with the period during which this moth infested the crop in San Lorenzo. In Majes and La Molina (like other coastal areas), farmers do not depend on the rain for irrigation, and they can sow quinoa at almost any time, so several generations of this moth may develop throughout the year in these valleys. Under this pattern of *E. melanocampta* incidence, designing pest management strategies for quinoa in the Andes is more feasible than in the non-traditional quinoa production zones, such as Majes and La Molina, unless farmers of the latter valleys take into account the organization of their sowing periods when setting up integrated pest management (IPM) schemes.

To better understand the impact of the incidence of *E. melanocampta* at the studied field sites, we refer to the economic threshold level of 3 to 15 larvae per plant, as suggested in previous studies (Blanco, 1994; Villanueva, 1978). Whereas in San Lorenzo, the infestation by this pest reached levels of up to 15 larvae per plant in 40 days (from 24/02/2016 to 4/4/2016), in La Molina, by only 21 days (from 17/11/2015 to 08/12/2016), even higher levels were attained (with up to 65 larvae per plant on average), exceeding, by far, the said threshold. According to Villanueva (1978), the occurrence of 30 larvae per quinoa plant may cause a 58.8% yield loss, whereas 70 larvae per plant could lead to an 85% loss.

One environmental factor that likely played a key role for *E. melanocampta* infestation is temperature. Previous observations pointed out that the pest's biological cycle is shortened from 75 to 28 days as the temperature increases from 20 to 24 °C (Quispe et al., 2014). In San Lorenzo, the mean monthly temperature oscillated between 14.4 and 15.3 °C, with large differences between the maximum and the minimum (up to 18 °C on average), which may have slowed down the development of the moth. Conversely, in La Molina, where the mean monthly temperature ranged from 19.4 to 21.6 °C (with maxima of up to 29.4 °C), the differences between the maximum and minimum temperatures did not exceed 7 °C, meeting the conditions for this pest to develop more generations throughout the cropping season; this may explain, in part, the higher incidence at this location as compared to San Lorenzo.

Aphids are considered secondary or occasional pests of quinoa in the Andes of Peru and Bolivia (Rasmussen et al., 2003), probably because their damage has been hard to pin down in terms of yield reduction or economic losses due to their overall low population density in the fields (Crespo & Saravia, 2014). The environmental variables in the highlands are often unfavourable for their population build up (i.e., rains, chilling temperatures and large differences between the minimum and maximum temperatures). For example, in San Lorenzo, the minimum temperature during the cropping season dropped to 0.1 °C, which is detrimental to aphid populations, which are considered in the chill-susceptible group, with "pre-freeze mortality" being the dominant cause of death at low temperatures (Bale et al., 1988). Contrariwise, the field site in La Molina had favourable conditions of temperature and relative humidity for the aphids to thrive (with up to 162 specimens per plant on average) (De Conti et al., 2011). With respect to Majes, the intensive use of insecticides during the first stages of the crop phenology and low incidence of the aphids at later stages did not allow revealing any such relation between climate and aphid populations.

Quinoa harbours an important diversity of natural enemies (Chapter 3), including aphidophagous insects (Valoy et al., 2015). However, this beneficial fauna is likely also affected by the unfavourable climate in San Lorenzo or the intensive insecticide treatments in Majes. These conditions appeared to have impaired the predatory group to a somewhat higher degree than the parasitoids, given that Aphidiinae wasps were collected in these two localities with parasitism levels of up to 13.5% in the first locality and 6.1% in the second, whereas the aphidophagous predators in San Lorenzo were scarce, and in Majes, they only developed once the pesticide spraying had finished. These observations could be explained, in part, due to the fact that the developed larvae of parasitoids inside the host integument are, to some degree,

protected from pesticide sprays, and part of the population inside the aphid mummy stage may experience a functional refuge (Sabahi et al., 2011).

In La Molina, more aphidophagous insects (in terms of abundance) were found than in the other two localities. A temporal succession in their occurrence was observed, which is related to their degree of feeding specialization: the aphid specialists (Aphidiinae wasps and predatory syrphid larvae) appeared in the early stages of infestation by *M. euphorbiae*, whereas the more generalist Chrysopidae larvae appeared at later stages (Campos & Sharkey, 2006; Thompson et al., 2010; Heckman, 2017). The effectiveness of these natural enemies, however, was likely perturbed by the insecticide applications. For example, the first spraying at 55 days after sowing with *B. thuringiensis* to control *E. melanocampta* may have had detrimental effects on *A. exotica* larvae, given that after this treatment, the increasing trajectory of their seasonal occurrence curve shifted to a decreasing trend, with a population reduction of around 42%. Although Horn (1983) found, on collards, that aphidophagous Syrphidae were reduced by a treatment with *B. thuringiensis* var. *kurstaki*, more studies are needed to clarify the potential risks of the use of *B. thuringiensis* for syrphid larvae.

The second treatment at the field site in La Molina with the insecticides dimethoate and methomyl was also detrimental to the syrphid larval population, likely due to both direct toxicity (Drescher & Geusen-Pfister, 1991) and a reduction in its aphid prey populations. Larval populations of chrysopids appeared after this insecticide treatment; being the predominant aphid predators at the later stages of the crop, they may have played an important role in keeping the aphids at a low density for some time after this spraying.

Thrips are also considered to be a secondary pest of quinoa, and there are no substantiated reports of significant yield reductions (Cranshaw et al., 1990; Crespo & Saravia, 2014). However, the seasonal occurrence patterns of *F. occidentalis* observed in Majes suggested that under favourable conditions, the thrips may infest the crop in an exponential way, reaching high levels of up to 191 thrips per plant on average. Considering that *F. occidentalis* possesses the basic characteristics for the fast development of pesticide resistance (a short generation time, high fecundity and haplodiploid breeding system) (Jensen, 2000), and pyrethroid insecticides are being widely used in Majes (Latorre, 2017), it is warranted to monitor the development of resistance in local populations of *F. occidentalis* to insecticides belonging to this chemical group. This would allow the implementation of proper insecticide resistance management by local farmers.

L. huidobrensis is another polyphagous pest that infested quinoa at relatively high densities (up to 3.36 larvae per plant) at the La Molina field site at mid stage of the crop phenology. The insecticide treatment on 09/11/2015 with dimethoate + methomyl markedly reduced the leafminer infestation. In the later stages of the crop, the temperature may have become less favourable (reaching up to 29 °C), preventing the pest from resurging. Previous studies indicate that high temperatures (25–30 °C) negatively influence the oviposition capacity of *L. huidobrensis* (Mujica et al., 2017). Conversely, the parasitoid complex of *L. huidobrensis* appears to be favoured by this range of temperatures (Sánchez & Redolfi de Huiza, 1988; Mujica et al., 2009; Burgos, 2013). Consequently, the seasonal occurrence of the parasitoids might have led to an effective control of the leafminer populations, with up to 100% parasitism (as the season became warmer), preventing *L. huidobrensis* from resurging. The occurrence of the parasitoid species in the field followed a similar pattern as in previous observations in potatoes in La Molina, where *Halticoptera* and *Chrysocharis* were the most abundant genera and, sporadically, *Diglyphus*, *Closterocerus* and *Ganaspidium* species were collected (Sánchez & Redolfi de Huiza, 1988).

L. hyalinus and *N. simulans* have been reported as infesting quinoa in large numbers in the departments of Lambayeque and Lima at the coastal level and in Arequipa in the “Maritime Yunga” zone of Peru (Cruces et al., 2016; Latorre, 2017). These hemipteran pests were observed causing severe damage to quinoa in the last months of 2013, throughout 2014 and in the first semester of 2015, during which some farmers admitted the overuse of pesticides even during the grain maturation stage (Latorre, 2017). Although no high level of infestation was registered in the present study, vigilance should be maintained, particularly when considering that the nymphs and adults of these true bugs cause direct damage to the grains by their piercing–sucking feeding habit during the grain filling and maturation stages, when management by applying insecticides increases the risk of residues on the harvested grains.

Producers may not be aware of *N. simulans* during the first stages of the crop because of its terrestrial behaviour, cryptic appearance and minute size. Moreover, the traditional way of harvesting quinoa, which involves leaving the cut plants on the ground for drying before threshing, favours *N. simulans* infestation. Another factor that promotes the pest’s incidence is its numerous host plants, encompassing a variety of crops and weeds, that allow them to find food in a wide variety of habitats (Cruces et al., 2016).

The strategy of pest control applied by the farmer at the field site in Majes followed a fixed schedule of treatments rather than a system based on the infestation level (the two first

spraying being performed every 7 days after sowing and the remaining three treatments, every 14 days). These insecticide applications occurred only during the first 60 days of the crop phenology, in order to reduce the risks of harvests being contaminated with chemical residues (E. Falconi, personal communication, May 2016, Majes). This management scheme appears to be used by most of the local quinoa growers, including also the recurrent use of pyrethroids (Latorre, 2017). This practice may be positive in terms of obtaining grains without residues, but the continuous use of active ingredients with the same mode of action (i.e., alpha-cypermethrin and zeta-cypermethrin) may eventually lead to the development of pesticide resistance in some of the key pests (Cisneros, 2012; Sparks & Nauen, 2015). Besides, the excessive use of broad-spectrum pesticides such as pyrethroids could cause harm to the environment (Hénault-Ethier, 2015) and have a negative impact on the natural enemy complex in quinoa (Croft & Whalon, 1982).

Conversely, the insecticide use in San Lorenzo was more appropriate, given that the treatments were performed once the pest reached a certain threshold. Besides, selective insecticides (*B. thuringiensis* + emamectin benzoate) were applied in a single treatment to control *E. melanocampta*. Nonetheless, this scheme does not reflect the general use of chemicals by farmers in the highlands growing conventional quinoa, who mainly use pesticides of the synthetic pyrethroid and organophosphate types (Rasmussen et al., 2003; Montoro et al., 2009; Latorre, 2017). Likewise, at the field site in La Molina, the pesticide treatments were also based on the infestation level of the pests; here, however, a mix of selective and non-selective insecticides were applied at a very high level of infestation. The pest management strategies deployed in the three localities suggest the continued need for agricultural extension programs in order to improve the use of agrochemicals.

The data gathered by on-plant and pitfall sampling suggests that the pest pressure in quinoa is higher at the lower altitudes than in the highlands of Peru. However, as crops are unstable systems, further research is needed to evaluate spatial and temporal variation of pests and their natural enemy populations in quinoa by sampling more fields and in different cropping seasons, more in particular in the new production zones of quinoa production where its pest complex has been poorly studied. In these areas there are better conditions for attaining higher yields than in the Andean region, pests are likely to become an important barrier for successful quinoa production, a situation that may worsen if pesticides are incorrectly used. These are issues that farmers from Peru, and other South American countries, will eventually face when exploiting new production areas. Studies on the biology and ecology of the key species of pests and their natural enemies will aid in implementing suitable pest control strategies for the crop.

Particularly, additional studies are needed to clarify the potential risks of aphids and *F. occidentalis* for quinoa production, especially in the non-traditional zones.

Chapter 5

Field evaluation of cypermethrin, imidacloprid, teflubenzuron and emamectin benzoate against pests of quinoa (*Chenopodium quinoa* Willd.) and their side effects on non-target species.

Redrafted after:

Cruces, L., de la Peña, E. and De Clercq, P. 2021. Field evaluation of cypermethrin, imidacloprid, teflubenzuron and emamectin benzoate against pests of quinoa (*Chenopodium quinoa* Willd.) and their side effects on non-target species. *Plants*, 10(9), 1788.

Author Contributions:

Conceptualization, L.C., E.d.I.P. and P.D.C.; methodology, L.C. and P.D.C.; formal analysis, L.C., E.d.I.P. and P.D.C.; investigation, L.C., E.d.I.P. and P.D.C.; data curation, L.C.; writing—original draft preparation, L.C.; writing—review and editing, L.C., E.d.I.P. and P.D.C.; supervision, E.d.I.P. and P.D.C.

5.1. Introduction

In the Andes, at an altitude between 2,300 and 3,800 m a.s.l., quinoa is traditionally cultivated since ancient times (Gamboa et al., 2018). In this region *Eurysacca melanocampta* Meyrick and *Eurysacca quinoae* Povolvý are the key pests of quinoa, causing damage by feeding on the developing grains; a range of other phytophagous insects are considered of minor importance (Saravia et al., 2014; Cruces et al., 2016).

At the coastal level, the number of relevant phytophagous insects infesting quinoa is substantially larger. These include species of wide distribution such as the cosmopolitan aphids (*Macrosiphum euphorbiae* (Thomas)), thrips (*Frankliniella occidentalis* (Pergande)) and leafminer flies (*Liriomyza huidobrensis* Blanchard); and also others of neotropical distribution such as certain true bugs (*Nysius simulans* Stål and *Liorhyssus hyalinus* (Fabricius)) and lepidopteran larvae (i.e., *E. melanocampta*, *Chloridea virescens* (Fabricius), *Spoladea recurvalis* Fabricius)) that feed on the developing grains (Cruces et al., 2016; Chapters 3 and 4). Under this scenario, farmers may be prompted to apply more pesticides than quinoa growers from the highlands. Hence the need for exploring a range of chemical compounds that may be suitable for use in an integrated pest management (IPM) program. As in other field crops (Torres & Bueno, 2018), selective insecticides with a more favourable toxicological profile to the natural enemy community may be a valuable tool for IPM in quinoa.

Cypermethrin is commonly used by the quinoa growers (Saravia et al., 2014). This insecticide of the pyrethroid group is a nonpersistent sodium channel modulator, characterized by a broad-spectrum activity. The compound acts by direct contact, causing neuronal hyperexcitation alongside the axon (Naumann, 2012; Latorre, 2017). Due to its relatively short residual effects and lower price, this pesticide is often overused, causing environmental issues and promoting resistance in pest insects (Desneux et al., 2007; Chapter 4). The adverse effects of cypermethrin on non-target organisms are widely documented (Desneux et al., 2007; Stanley & Preetha, 2016).

Imidacloprid is one of the most widely used insecticides worldwide (Zhu et al., 2017a, 2017b; Dadther-Huaman et al., 2020) and it is also commonly used by farmers in coastal areas of quinoa production (Cáceres del Carpio & Iannacone, 2021). This compound may effectively control a range of phytophagous insects noted to be pests of quinoa (including aphids, thrips, true bugs and some lepidopteran species). This neurotoxic insecticide of the neonicotinoid group, is an acetylcholine receptor agonist with broad spectrum and highly systemic activity, acting by

ingestion and direct contact, causing neuronal hyperexcitation at the level of the synapses (Jeschke et al., 2019; Chapter 4). Toxicity of imidacloprid to non-target organisms, including beneficial species such as pollinators (i.e. bees) and natural enemies has been documented in different crops, but there are presently no reports for quinoa (Desneux et al., 2007; Prabhaker et al., 2011; El-Naggar & Zidan, 2013; Douglas & Tooker, 2016; Stanley & Preetha, 2016; Calvo-Agudo et al., 2019; Motaung, 2020; Ricupero et al., 2020). Due to these adverse effects, particularly to the bees, this active ingredient has been banned in Europe (Gasparic et al., 2020).

Teflubenzuron has a more favourable environmental profile, with lesser toxicity to a range of non-target organisms as compared to the broad-spectrum compounds, and thus may be considered as a tool for an IPM program in quinoa (Jeschke et al., 2019). This insect growth regulator (IGR) of the benzoylphenylureas group is a highly active inhibitor of chitin synthesis, aimed mainly at lepidopteran larvae. This compound is considered to be safer to the beneficial fauna (especially in the adult stage) than pyrethroids and neonicotinoids, although there are reports of its toxicity towards a number of arthropod predators (Ishaaya & Degheele, 1998; Stanley & Preetha, 2016; El-Wakeil et al., 2013; Spomer & Sheets, 2019).

Emamectin benzoate, a neurotoxic insecticide of the avermectin group, is another insecticide reported to be more selective against lepidopteran larvae. Although toxicity to some natural enemies and non-target arthropods have been reported, this insecticide is considered less harmful to beneficial arthropods as compared with broad spectrum compounds (European Food Safety Authority, 2012; Stanley & Preetha, 2016). The insecticide acts mainly by ingestion causing paralysis in the insect by activating allosterically the glutamate-gated chloride channels in the synapses (Jansson & Dybas, 1998; Ishaaya et al., 2007; El-Wakeil et al., 2013; Stanley & Preetha, 2016; Pitterna, 2019).

The aim of the present field study was to examine the effects of two broad spectrum insecticides (cypermethrin and imidacloprid) and two selective insecticides (teflubenzuron and emamectin benzoate), with different modes of action and from different chemical groups, against quinoa pests in Peru and record their side effects on non-target arthropods by analysing the species composition, species diversity and population density in quinoa fields at the coastal level (a region with potential areas for quinoa production). To assess the effects of these insecticides on the arthropod community, including phytophagous and beneficial species, we combined three sampling techniques (i.e., pitfall traps, plant sampling and yellow pan traps) targeting groups from different ecological habitats. The findings of this field study should be of special interest to quinoa farmers and agricultural extensionists from Andean and non-Andean countries who are

exploring the cultivation of quinoa, to use insecticides of higher compatibility with natural biological control as part of an IPM approach.

5.2. Materials and Methods

5.2.1. Location

The study was carried out in experimental fields belonging to the National Agrarian University La Molina in Lima, Peru (coordinates: 12°04'57.0''S, 76°56'49''W; altitude: 244 m).

5.2.2. Experimental plots

The field trial was conducted under a stratified–randomized design with three replications. Each experimental plot consisted of 21 m² (7 ridges of 0.75 m width, 4 m length), with a plant density of 36 quinoa plants per linear meter (variety “Negra Collana”) after seedling removal. Each plot (as shown in Figure 5.1) was surrounded by polypropylene films (0.5 mm thickness, 1.5 m height, black colour) three days before the treatment (15/09/2017) and maintained until the time of harvest. At the beginning of the grain filling stage (on 05/11/2017), the whole experiment was covered with antibird netting to protect the crop from bird damage. Growing specifications of the field site are described in Table 5.1.

5.2.3. Insecticide treatments

Treatments were done with four insecticides (as formulated materials): teflubenzuron (150 g/l), emamectin benzoate (50 g/kg), imidacloprid (350 g/l) and cypermethrin (250 g/l). Water was used as a negative control. Specifications of the insecticides are described in Table 5.2.

The insecticides were applied using a manual sprayer (SOLO461: pressure 3 bars, capacity 5 L) with a full cone nozzle (TeeJet®). The sprayer was calibrated to apply 0.65 L per plot (corresponding to 300 L per ha). Before insecticide dissolution, water was acidified to a pH range of 5.0 to 6.0 (as recommended on the labels), with an acidifying product (SUPER ACID, 43% of organic and inorganic acids) at 0.05%. Similarly, water was acidified for the negative control.

Two applications at the maximum recommended field rate (Table 5.2) were made at flowering stage on 18/09/2017 (61 days after sowing) and 03/10/2017 (76 days after sowing). At this crop phase, the plants had reached their maximum height (ca., 1.2 m). The top of the plants and their sides were treated.

Table 5.1. Growing specifications of the experimental field.

	Specifications	Dates
Sowing	drilling sowing method	19/07/2017
Harvest	threshing	12/12/2017
Irrigation	surface irrigation	(20/07/2017; 17/08/2017; 07/09/2017; 28/09/2017; 25/10/2017; 09/11/2017)
Fertilisation doses (NKP)	160–80–160	19/07/2017
Soil type	clay loam	-
Neighbouring crops	Quinoa (<i>Chenopodium quinoa</i>), Wheat (<i>Triticum</i> spp.) Corn (<i>Zea mays</i>) Kiwicha (<i>Amaranthus caudatus</i>)	-
Fungicides	1° benzomyl 2° metalaxyl + mancozeb 3° dimetomorph	(24/07/2017) (07/08/2017) (25/08/2017)
Weed management	Manual control	(25/07/2017, 11/08/2017; 19/11/2017)
Previous crop	Fallow period of 4 months	-

Table 5.2 Insecticide specifications used in the treatments.

Insecticide	Label field rate (g a.i. ha ⁻¹) *	Chemical group	Commercial name	Company
Cypermethrin	75	Pyrethroid	Cypmor 25 EC	Jebsen and Jessen Peru S.A.C.
Teflubenzuron	33.75	Benzoylphenylurea	Mercury 150 SG	Point Andina S.A.
Emamectin benzoate	10	Avermectin	Olimpo 5% SG	Sharda Peru S.A.C.
Imidacloprid	131.25	Neonicotinoid	Phantom	Jebsen and Jessen Peru S.A.C.

a.i. = active ingredient.

*spray liquid applied at a rate of 300 L/ha.

5.2.4. Sampling methodology

Three sampling techniques were used for studying the arthropod fauna (insects and arachnids): pitfall trapping, for ground dwelling species; plant sampling, for foliage dwelling species (phytophagous insects and natural enemies); and, pan traps placed at the level of the top canopy (1.2 m), for flying insects.

5.2.4.1. Pitfall trapping

One pitfall trap (as an experimental unit) was installed in the middle of each experimental plot six days after the first insecticide application and maintained until one day before harvest (from

24/09/2017 to 11/12/2017). Traps consisted of a polypropylene container (transparent, \varnothing 10 cm at opening and at bottom, 12 cm deep) with a mix of water and 40% v/v formaldehyde (9:1), and a few drops of detergent. The pitfall trap content was periodically collected (a total of 5 times) in airtight recipients (of the same dimensions as the traps) and carefully labelled to be transported to the laboratory for further processing. Thereafter, the collection fluid was replaced.

5.2.4.2. Plant sampling

At each experimental plot, four samplings were performed, i.e., one day before the first application (17/09/2017), 6 days after the first application (24/09/2017), 6 days after the second application (09/10/2017) and 69 days after the second application (11/12/2017). Sampling consisted of taking three plants from crop rows 3 and 6 (Figure 5.1) in the 1st and 3rd samplings, and from rows 2 and 5 in the 2nd and 4th samplings. Plants near the borders of the plots were always avoided.

In each plot, every plant was randomly selected and carefully collected: the plant (after cutting it at its base with scissors) was shaken over a container (width 26 cm \times large 36 cm \times height 20 cm) with a mix of water and 96% v/v ethanol (3:1), and some drops of liquid detergent. Thereafter, the sampled plants were carefully chopped into small pieces and the whole sample (including the liquid content) was transferred to an airtight container (volume 3l).

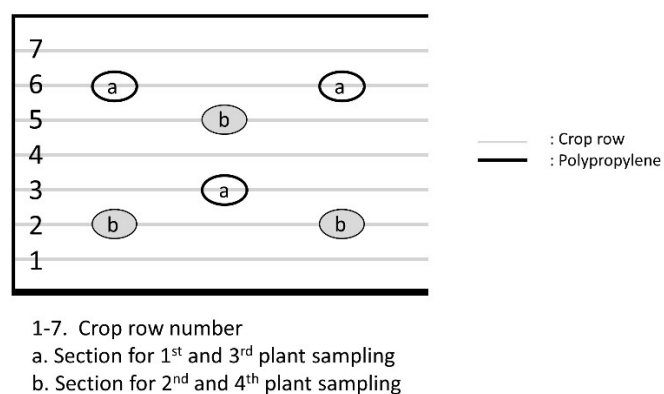


Figure 5.1. Schematic diagram of plant sampling setup for an experimental plot.

5.2.4.3. Pan trapping

Each pan trap consisted of a yellow polypropylene container (\emptyset 20 cm at opening and 18 cm at bottom, 7 cm deep) with a mix of water and 40% v/v formaldehyde (9:1), and some drops of detergent. One pan trap was installed in the middle of each experimental plot, at a height of 1.2 m (the opening at the level of the top of the crop canopy), six days after the first insecticide application and maintained until one day before harvest (from 24/09/2017 to 11/12/2017). As these traps were exposed to desiccation, they were regularly inspected and when needed, they were refilled with the same collection fluid.

The pan trap content was periodically collected (5 times) in airtight recipients (500 ml of capacity) and carefully labelled to be transported to the laboratory for further processing. Thereafter, the collection fluid was replaced.

5.2.5. Sample processing

All samples were processed at the laboratories of the Museum of Entomology “Klaus Raven Büller” of the National Agrarian University La Molina, in Lima, Peru, where the collected specimens were deposited.

5.2.5.1. Sample washing

The recipients containing pitfall trap and pan trap samples were poured onto a 1 mm mesh sieve and carefully washed with water, removing larger material such as stones, straw or leaves. The collected specimens were transferred to a labelled plastic container (\emptyset 5 cm, 6 cm length) containing 75% v/v ethanol for conservation and further processing (i.e., morphotyping).

The recipient with the plant samples was decanted through a 1 mm mesh sieve and carefully washed. Then, the plant parts (leaves, stem and panicle) were examined under a binocular stereoscope (Carl Zeiss: Stemi 508) to check for the presence of mines and to collect the insects that remained stuck to the plant. These specimens and those which easily detached from the plant materials were transferred to a labelled plastic container (\emptyset 5 cm, 6 cm length) containing 75% v/v ethanol for conservation and morphotyping.

5.2.5.2. Morphological identification

The specimens were examined using a binocular stereoscope (Carl Zeiss: Stemi 508) and sorted on the basis of morphological characteristics as morphospecies (Oliver & Beattie, 1993, 1996). Each new morphospecies was photographed and codified, facilitating comparison when a new

similar morphospecies was found, and then placed in a glass vial (\emptyset 1.5 cm, 4 cm length) with 75% v/v ethanol for preservation. When necessary, the morphotypes were re-examined. Each morphospecies was counted and classified at family level with the help of taxonomic keys from the literature (Triplehorn & Johnson, 2005).

The most abundant morphospecies were identified to genus level and, when possible, to level species with a help of specific taxonomic keys as follows: *Blennidus peruvianus* Dejean (Moret, 1995, 2003); *L. hyalinus* (Göllner-Scheiding, 1976); *N. simulans* (Pall et al., 2016), *L. huidobrensis* (Korytkowski, 2014; Spencer, 1973), *Rhinacloa* sp. (Hernández & Henry, 2010), *Metacanthus tenellus* Stål (Gross, 1950; Henry et al., 2015); *Nabis capsiformis* (Germar) (Kerzhner & Henry, 2008; Cornelis & Coscarón, 2013; Cornelis, 2015); *S. recurvalis* (Solis, 2006). The identification of the Araneae families and genera (i.e., *Laminacauda* sp.) was assisted by arachnologist Manuel Andía associated to the Museum of Entomology “Klaus Raven Büller”.

5.2.5.3. Molecular identification

DNA extraction and PCR procedures were performed at the Department of Plants and Crops of Ghent University in Belgium to identify and/or confirm the species *L. huidobrensis*, *M. euphorbiae*, *L. hyalinus* and *F. occidentalis*, following the protocols provided in the literature (Shufran & Puterka, 2011; Nakamura et al., 2013; Harbhajan & Kaur, 2017; T. Ding et al., 2018; Nguyen et al., 2019).

DNA was extracted from a single specimen (*M. euphorbiae*, *F. occidentalis*) or a leg (*L. hyalinus*, *L. huidobrensis*) that was removed from an adult specimen with a fine cutter. The sample was transferred to a 1.5 ml Eppendorf and then crushed with a plastic rod with 20 μ L of STE-buffer (100 mM NaCl, 10 mM Tris-HCL, 1 mM EDTA, pH 8.0) and 2 μ L of proteinase K (10 mg/mL). This mix was incubated at 60 °C for 30 min. Then, the activity of the proteinase K was stopped at 95 °C for 5 min.

DNA samples were subjected to PCR analysis with the primers LCO1490 FW and HCO2198RV (for *L. hyalinus* and *L. huidobrensis*); MTD 7.2 F and COI-MTD 9.2 R (for *F. occidentalis*); and, C1-J-1718 and C1-N-2191 (for *M. euphorbiae*). Amplification was performed in 50 μ L total mix reaction, containing 2 μ L of DNA sample, 0.25 μ L GoTaq® DNA Polymerase (5 u/ μ L), 3 μ L MgCl₂ solution (25 mM), 1 μ L dNTPs (10 μ M each), 2.5 μ L forward primer, 2.5 μ L reverse primer, 10 μ L: 5 \times Colorless GoTaq® Flexi Buffer, 28.75 μ L water. This solution was placed in a thermal cycler with the following parameters: 2 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 66 °C, 45 s at 72 °C, and a final extension of 5 min at 72 °C. After amplification, 10 μ L of the PCR products were

subjected to electrophoresis on a 2% agarose gel, and PRC products were purified using the EZNA[®] Cycle Pure Kit (Omega BioTek) following the manufacturer's protocols. Bidirectional Sanger sequencing, using the PCR primers, was outsourced to LGC genomics (Germany).

5.2.6. Statistical analysis

For each sampling methodology applied, differences in the collected species composition between treatments were evaluated with the Bray–Curtis dissimilarity index and NMDS (non-metric multidimensional scaling) using the presence and abundance of the morphospecies to detect distances between plots. Significant differences between treatments were assessed using the PerMANOVA test (999 permutations).

The effects of the insecticide treatments on the diversity of the arthropod community, per each sampling methodology, were analysed through a) the rank abundance curves and the indices of Shannon and Simpson's dominance to evaluate the structure of the community (evenness and dominance of species) and b) the Margalef index to assess the species richness. They were calculated for each experimental plot.

The diversity indices, mean numbers of the major pests and mean numbers of natural enemies were compared, according to each sampling methodology, between treatments by one-way ANOVA and Duncan tests, after having tested the normality and homoscedasticity of the data through Shapiro–Wilk and Bartlett tests, respectively. When the data did not meet the assumption of homogeneity of variances, the Box–Cox transformation method was applied to stabilize the variance; however, untransformed data are presented in the tables.

All statistical analyses were performed using R software, version 3.4.2 (R Core Team, 2020). The tests were analysed at a significance level of $\alpha = 0.05$.

5.3. Results

5.3.1. Effects on the composition of the arthropod fauna

The NMDS-plots show the distances between treatments concerning the composition of the arthropods collected from 24/09/2017 to 11/12/2017, with the different sampling methodologies (in Figure 5.2), based on the Bray–Curtis dissimilarity index. Ellipses are formed by the replications of each treatment (based on the presence and abundance of species) and the closeness or distances between them reflect their similarities and dissimilarities, respectively. Some morphospecies are shown in the NMDS-plots to depict the differences in the

species composition collected in each insecticide treatment: the distance of a morphospecies to the centre of an ellipse (treatment) reflects its scarcity or even absence in the treatment.

As to the pitfall trap data, the proximity of the ellipses in the NMDS-plot (Figure 5.2 a) suggests high similarity among the treatments in terms of the composition of ground dwelling species, which was confirmed by the PerMANOVA test that indicated no significant differences between treatments ($F - model_{4,10} = 0.7537, p = 0.819$). Likewise, for the flying insects collected at the top of the canopy with the pan traps (Figure 5.2 c), no significant differences between treatments were found ($F - model_{4,10} = 1.066, p = 0.441$). For the plant sampling data (i.e., the specimens collected from the quinoa plants), however, the ellipse corresponding to the imidacloprid treatment in the NMDS-plot (Figure 5.2 b) is separated from the others, suggesting high dissimilarity between imidacloprid and the other treatments; in this case, the test was significant ($F - model_{4,10} = 2.835, p = 0.001$).

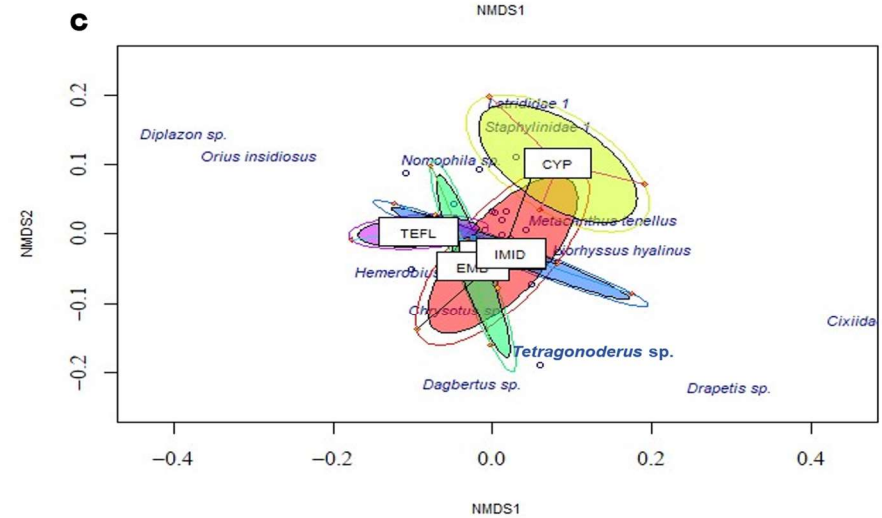
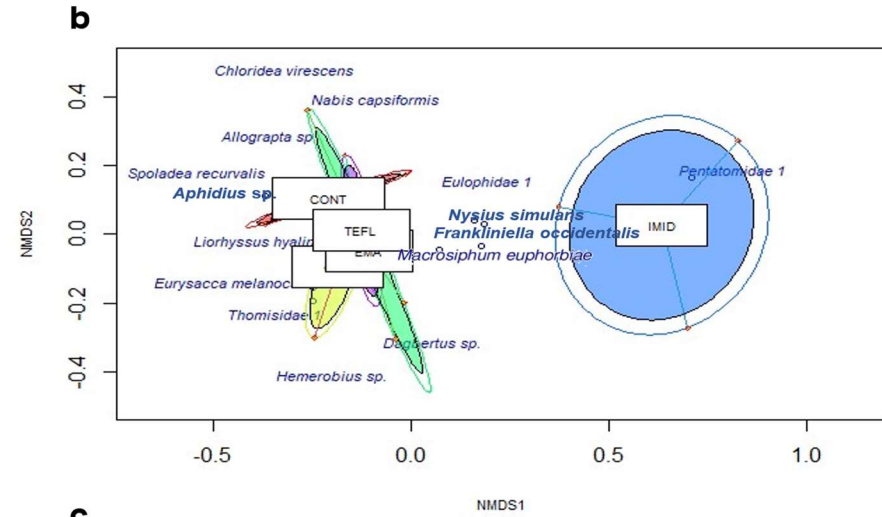
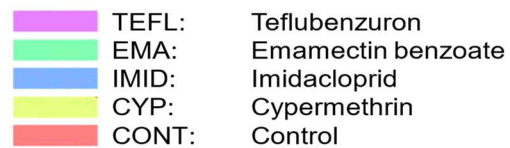
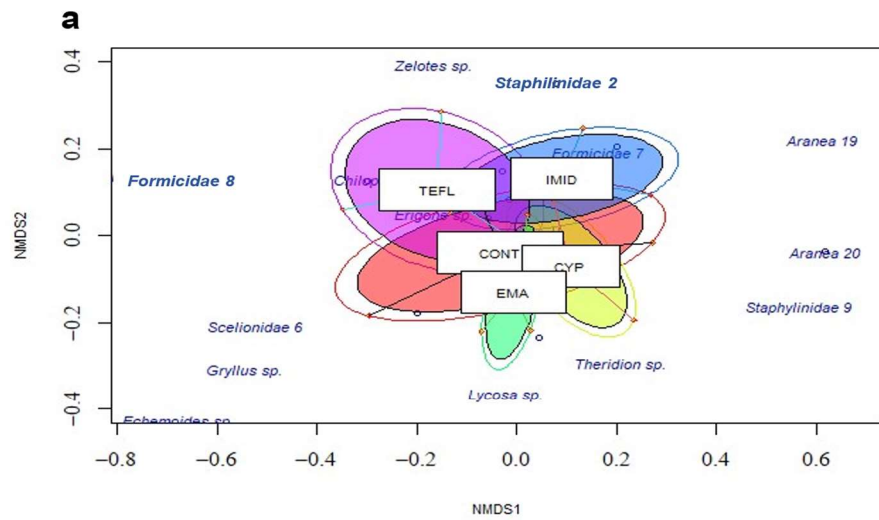


Figure 5.2. The NMDS plot showing the compositional distance between treatments for different sampling methods: a. pitfall trapping; b. plant sampling; c. pan trapping. Plots are displayed by orange dots; plots that belong to the same treatment are fitted in a single ellipse. Each treatment is represented by a different colour.

5.3.2. Effects on diversity of arthropods

5.3.2.1. Structure

Rank abundance curves of the morphospecies collected in the period between 24/09/2017 and 11/12/2017, were calculated for each insecticide treatment (Figure 5.3). For the pitfall trap data, the corresponding curves for the treatments and the untreated control have similar patterns, except for imidacloprid in which a slightly more pronounced slope can be observed (Figure 5.3 a), indicating that the imidacloprid treatment affected the evenness of the ground dwelling arthropod community to a higher degree than the other insecticides. When applying the ANOVA, significant differences between treatments were found for the Shannon ($F_{4,8} = 4.109, p = 0.042$) and Simpson's dominance ($F_{4,8} = 4.038, p = 0.038$) indices. The Duncan test confirmed that imidacloprid had a significantly greater impact on the species equitability (with lowest value of Shannon index), preventing dominance of certain taxa (with the lowest value of Simpson's dominance index) (Table 5.3).

As to the specimens collected from the quinoa plants, the corresponding curve for imidacloprid markedly differs from the other treatments and the untreated control, due to the lower number of species collected (Figure 5.3 b). However, no significant differences between treatments for the Shannon ($F_{4,8} = 2.57, p = 0.119$) and Simpson's dominance ($F_{4,8} = 1.81, p = 0.220$) indices were found (Table 5.3). For the pan trap data, the curves for all treatments and the control have similar patterns (Figure 5.3 c), suggesting a similar distribution of flying species in the community over all plots. The ANOVA confirmed no significant differences between treatments in terms of the Shannon ($F_{4,8} = 0.34, p = 0.841$) and Simpson's dominance ($F_{4,8} = 0.45, p = 0.771$) indices.

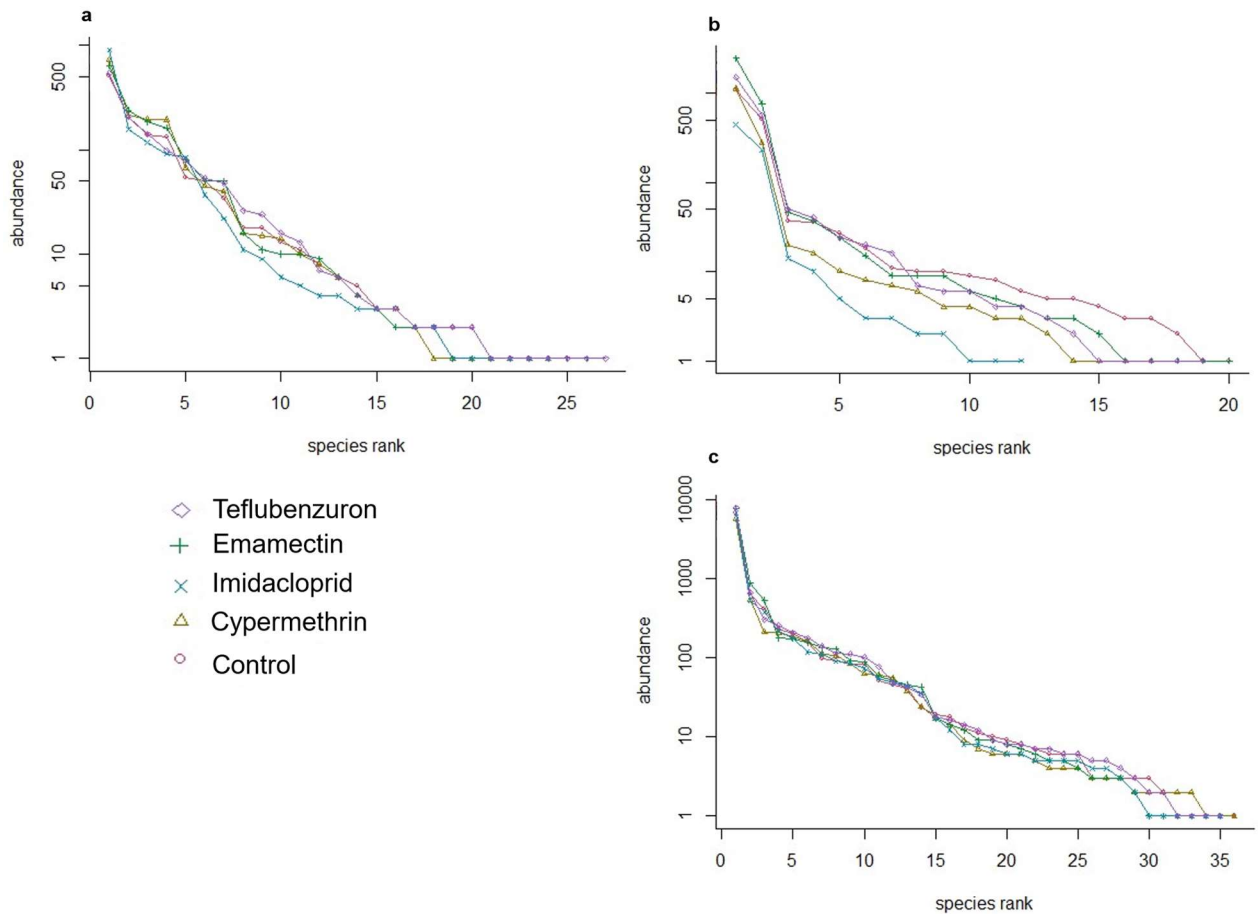


Figure 5.3. Rank abundance curves for the morphospecies found with the different sampling methodologies, per treatment (log series distribution): a. pitfall trapping; b. plant sampling; c. pan trapping.

5.3.2.2. Species richness

Significant differences between treatments were found in species richness of the ground dwelling arthropods, measured by the Margalef index ($F_{4,8} = 5.55, p = 0.019$). The lowest species richness was obtained with imidacloprid, being significantly inferior to that of the teflubenzuron and emamectin benzoate treatments and the untreated control, but without significant differences with cypermethrin. Significant differences were also found for the insects collected from the plants ($F_{4,8} = 4.76, p = 0.029$), with the imidacloprid treatment having the lowest value. For the pan trapping data, no significant differences in species richness of flying insects between treatments and the control were found ($F_{4,8} = 0.49, p = 0.747$) (Table 5.3).

Table 5.3. Diversity index (mean \pm standard deviation) of morphospecies, according to the sampling methodology applied, collected after the first insecticide treatment (sampling period between 24/09/2017 and 11/12/2017).

Diversity index	Treatments					F value	p-value
	Teflubenzuron	Emamectin Benzoate	Imidacloprid	Cypermethrin	Control		
<u>Pitfall trapping</u>							
Shannon	1.92 \pm 0.11 ^a	1.80 \pm 0.01 ^a	1.44 \pm 0.11 ^b	1.73 \pm 0.17 ^a	1.87 \pm 0.25 ^a	4.11	0.042
Simpson's dominance	0.77 \pm 0.03 ^a	0.75 \pm 0.05 ^a	0.60 \pm 0.05 ^b	0.73 \pm 0.09 ^a	0.76 \pm 0.09 ^a	4.04	0.038
Margalef	3.06 \pm 0.16 ^a	2.85 \pm 0.09 ^{ab}	2.48 \pm 0.12 ^c	2.68 \pm 0.21 ^{bc}	2.78 \pm 0.16 ^{ab}	5.55	0.019
<u>Plant sampling</u>							
Shannon	0.99 \pm 0.08 ^a	0.84 \pm 0.15 ^a	0.94 \pm 0.18 ^a	0.82 \pm 0.13 ^a	1.13 \pm 0.06 ^a	2.57	0.119
Simpson's dominance	0.49 \pm 0.06 ^a	0.43 \pm 0.09 ^a	0.52 \pm 0.10 ^a	0.40 \pm 0.08 ^a	0.55 \pm 0.02 ^a	1.81	0.220
Margalef*	1.71 \pm 0.13 ^a	1.71 \pm 0.26 ^a	1.09 \pm 0.27 ^b	1.78 \pm 0.06 ^a	1.97 \pm 0.52 ^a	4.76	0.029
<u>Pan trapping</u>							
Shannon	1.11 \pm 0.14	1.13 \pm 0.19	1.09 \pm 0.01	1.18 \pm 0.10	1.13 \pm 0.05	0.34	0.841
Simpson's dominance	0.40 \pm 0.06	0.43 \pm 0.08	0.04 \pm 0.01	0.44 \pm 0.04	0.42 \pm 0.04	0.45	0.771
Margalef*	3.52 \pm 0.29	3.40 \pm 0.08	3.50 \pm 0.20	3.61 \pm 0.15	3.61 \pm 0.23	0.49	0.747

Different letters within a row indicate significant differences at $\alpha = 0.05$ (Duncan test), when the ANOVA was significant.

*ANOVA run after using Box-Cox transformation method ($\gamma = 0.2$).

5.3.3. Effects on functional species pools

5.3.3.1. Phytophagous group

Four herbivorous species infested the plots in relatively high abundance: *S. recurvalis*, which appeared at the early stages of the crop phenology and *M. euphorbiae*, *F. occidentalis* and *N. simulans*, the incidence of which in all plots was recurrent throughout the cropping season. The mean numbers of these species per plant were compared between treatments (Table 5.4).

The statistical analysis indicated that all insecticides were efficient to reduce *S. recurvalis* incidence after the first application as compared to the untreated control ($F_{4,8} = 7.73, p = 0.007$). Since this pest had disappeared in the treated plots, the effects after the second application could not be evaluated, neither at day 6 nor at day 69 after the application.

Significant differences in the numbers of *M. euphorbiae* were observed, 6 days after the first ($F_{4,8} = 28.73, p < 0.001$) and 6 days after the second applications ($F_{4,8} = 7.32, p = 0.008$), and also 69 days after the second application ($F_{4,8} = 7.80, p = 0.007$). Six days after the first application, the lowest mean number per plant was obtained with imidacloprid, differing significantly from the numbers observed in the teflubenzuron and emamectin benzoate treatments and in the untreated control, whereas the imidacloprid and cypermethrin treatments were similar. On day 6 after the second application, significantly lower aphid numbers were registered for the imidacloprid and cypermethrin treatments, whereas the teflubenzuron and emamectin benzoate treatments were similar to the untreated control. At the last sampling, 69 days after the second application, the aphid numbers were similar in the teflubenzuron, cypermethrin and the untreated plots; the highest aphid abundance was recorded with emamectin benzoate and the lowest with imidacloprid.

Significant differences in *F. occidentalis* numbers were observed 6 days after the first ($F_{4,8} = 9.47, p = 0.004$) and 6 days after second applications ($F_{4,8} = 171.17, p < 0.001$), and 69 days after the second application ($F_{4,8} = 46.76, p < 0.001$). On day 6 after the first application, the lowest mean values were observed with imidacloprid and cypermethrin, and no significant differences were found between teflubenzuron, emamectin benzoate and the untreated control. Six days after the second application, the lowest mean values were also obtained with imidacloprid and cypermethrin, and lower thrips numbers were found in the teflubenzuron and emamectin benzoate plots as compared to the untreated control. At the last sampling date, 69 days after the second application, the lowest thrips numbers were obtained with imidacloprid, whereas the cypermethrin and teflubenzuron treatments and the untreated control had similar

numbers; the thrips numbers in the emamectin benzoate plots were significantly higher than in the other plots.

Significant differences in *N. simulans* numbers were not seen until 69 days after the second application ($F_{4,8} = 25.87, p < 0.001$). The lowest mean values were obtained with imidacloprid and cypermethrin (in this order); the mean value obtained in the untreated control was similar to that in the cypermethrin and emamectin benzoate treatments, whereas the highest value was recorded in the teflubenzuron treatment.

The phytophagous insects were also examined in the pitfall and pan traps. The mean cumulative number of *N. simulans* and *F. occidentalis*, recorded from 24/09/2017 to 11/12/2017, were compared between treatments (Table 5.5). No significant differences between treatments were found as to *N. simulans* numbers trapped with pitfall traps ($F_{4,8} = 1.62, p = 0.261$) nor as to those collected with pan traps ($F_{4,8} = 0.42, p = 0.792$).

There were significant differences in *F. occidentalis* numbers collected with pan traps ($F_{4,8} = 11.18, p = 0.002$). The lowest mean values were observed in the cypermethrin treatments, whereas the highest thrips numbers were noted in the teflubenzuron plots; the emamectin benzoate, imidacloprid and untreated plots had similar values. Adults of *L. huidobrensis* were also collected in relatively high abundance in the pan traps, but as they appeared at the later stages of the crop phenology, leafminer larvae were not observed in the examined leaves and therefore they were not considered in the analysis.

5.3.3.2. Natural enemies

The most recurrent natural enemy groups found on the collected plants were Aphidiinae wasps (adult and parasitized aphids), predatory true bugs (*M. tenellus*, *Rhinacloa* sp., and *N. capsiformis*), syrphid larvae (*Allograpta* sp.) and chrysopid larvae. The mean cumulative numbers of individuals per plot of these groups were compared between treatments (Table 5.6). Other predators such as coccinellids and hemerobiids were also found in some plots, but their incidence was irregular throughout the monitoring and in small numbers; therefore, they were excluded from the analysis.

Numbers of Aphidiinae wasps collected in the plants did not differ between treatments, neither 6 days after the first application ($F_{4,8} = 2.66, p = 0.111$), nor 6 days after the second application ($F_{4,8} = 2.12, p = 0.169$). However, 69 days after the second application, significant differences were found ($F_{4,8} = 4.07, p = 0.043$), with zero specimens of Aphidiinae wasps

(neither larvae in the mummified aphids nor adults) collected in the plots treated with imidacloprid.

The predatory heteropterans were scarce 6 days after the first and the second applications (with only seven specimens recorded over the different plots) and data recorded in these samplings were not subjected to ANOVA. On day 69 after the second application, the predatory true bugs became relatively more abundant, and significant differences between treatments and the control ($F_{4,8} = 5.48, p = 0.020$) were found, with the lowest numbers of heteropterans predators recorded in the imidacloprid and cypermethrin treatments, whereas values for the untreated control and the teflubenzuron and emamectin benzoate plots were similar.

No syrphid larvae were observed in the plots before the first application. Whereas six days after the first application, numbers of syrphid larvae did not differ among treatments ($F_{4,8} = 3.09, p = 0.082$), significant differences were found 6 days after second application ($F_{4,8} = 5.39, p = 0.021$), with zero specimens collected in the imidacloprid, cypermethrin and teflubenzuron treatments.

Since chrysopid larvae appeared at the later stages of the crop phenology, short-term effects of the insecticides on their numbers could not be observed. However, 69 days after the second application, significant differences in numbers of chrysopid larvae between the insecticide treatments and the untreated control were observed ($F_{4,8} = 4.35, p = 0.037$), with zero specimens collected in the imidacloprid plots, and very low numbers as well in the cypermethrin and emamectin benzoate plots. Incidence of chrysopid larvae in the teflubenzuron treatment was similar to that in the control.

Table 5.4. Numbers of individuals of the major insect pests (mean no. per plant \pm standard deviation) under different treatments.

Taxa	Treatments					F value	p-value
	Teflubenzuron	Emamectin Benzoate	Imidacloprid	Cypermethrin	Control		
<i>Spoladea recurvalis</i>							
1DBA	2.4 \pm 1.39	2.8 \pm 0.51	2.7 \pm 0.33	3.2 \pm 1.64	2.4 \pm 1.02	0.15	0.956
<i>1st application</i>							
*6DAA	0.4 \pm 0.77 ^b	0.1 \pm 0.19 ^b	0 \pm 0.0 ^b	0.1 \pm 0.19 ^b	2.0 \pm 0.88 ^a	7.73	0.007
<i>2nd application</i>							
6DAA	0	0	0	0	0.67 \pm 0.67	N.A.	N.A.
69DAA	0	0	0	0	0	N.A.	N.A.
<i>Macrosiphum euphorbiae</i>							
1DBA	11.4 \pm 4.33	15.7 \pm 9.17	13.1 \pm 4.19	20.4 \pm 10.49	12.3 \pm 4.26	1.33	0.338
<i>1st application</i>							
**6DAA	11.6 \pm 4.74 ^b	7.8 \pm 0.77 ^b	1.8 \pm 2.04 ^c	2.9 \pm 2.99 ^c	27.4 \pm 10.83 ^a	28.73	< 0.001
<i>2nd application</i>							
6DAA	6.9 \pm 4.44 ^a	11.2 \pm 3.56 ^a	0.2 \pm 0.19 ^b	0.3 \pm 0.33 ^b	6.6 \pm 3.89 ^a	7.32	0.008
***69DAA	145 \pm 40.19 ^{ab}	250 \pm 104.46 ^a	36.2 \pm 1.67 ^c	113.8 \pm 25.06 ^b	86.1 \pm 27.48 ^{bc}	7.80	0.007
<i>Frankliniella occidentalis</i>							
1DBA	2.2 \pm 1.26	2.4 \pm 1.26	2.9 \pm 0.84	4.3 \pm 0.58	1.8 \pm 0.68	2.62	0.115
<i>1st application</i>							
6DAA	5.3 \pm 1.20 ^a	4.7 \pm 1.15 ^a	2.3 \pm 0.33 ^b	1.4 \pm 0.38 ^b	5.4 \pm 1.17 ^a	9.47	0.004
<i>2nd application</i>							
***6DAA	5.1 \pm 0.84 ^b	4.3 \pm 1.15 ^b	2.1 \pm 0.51 ^c	0.6 \pm 0.19 ^c	9.89 \pm 2.46 ^a	171.17	< 0.001
69DAA	26.3 \pm 11.1 ^b	62.1 \pm 6.50 ^a	7.2 \pm 1.89 ^d	15.1 \pm 5.42 ^{bc}	20.3 \pm 11.98 ^{bc}	46.76	< 0.001
<i>Nysius simulans</i>							
*1DBA	0.1 \pm 0.19	0.2 \pm 0.19	0.1 \pm 0.19	0.3 \pm 0.33	0.8 \pm 0.84	0.82	0.549
<i>1st application</i>							
6DAA	0 \pm 0	0 \pm 0	0.2 \pm 0.19	0.1 \pm 0.19	0.1 \pm 0.19	1.75	0.232
<i>2nd application</i>							
*6DAA	0.6 \pm 0.38	0.6 \pm 0.69	0.3 \pm 0.33	0.1 \pm 0.19	0 \pm 0	1.62	0.261
69DAA	5.0 \pm 0.67 ^a	3.6 \pm 0.19 ^b	1.0 \pm 0.67 ^d	2.0 \pm 0.33 ^c	2.9 \pm 0.19 ^{bc}	25.87	< 0.001

DBA: days before application; DAA: days after application; N.A.: not applicable.

Different letters within a row indicate significant differences at $\alpha = 0.05$ (Duncan test), when the ANOVA was significant.

*ANOVA run after using Box–Cox transformation method $\gamma = -2.0$, ** $\gamma = 0.45$, *** $\gamma = 0.30$.

The natural enemies were also examined in the pitfall traps. The most abundant morphospecies collected from 24/09/2017 to 11/12/2017 were the arachnid *Laminacauda* sp., the ground beetle *B. peruvianus* and the wasp *Trimorus* sp.; the mean cumulative number of these morphotypes was compared between treatments and control (Table 5.5). There were no significant differences between treatments in numbers of *Laminacauda* sp. ($F_{4,8} = 2.21, p = 0.159$), *B. peruvianus* ($F_{4,8} = 0.061, p = 0.669$) and *Trimorus* sp. ($F_{4,8} = 0.78, p = 0.568$).

When examining the natural enemies collected in the pan traps (recorded from 24/09/2017 to 11/12/2017), three groups were the most abundant: Dolichopodidae, Syrphidae and Aphidiinae. There were no significant differences between treatments in the numbers of adults of Dolichopodidae ($F_{4,8} = 1.14, p = 0.403$) and Syrphidae ($F_{4,8} = 3.33, p = 0.069$). The number of Aphidiinae wasps was similar when comparing each treatment with the untreated control, but in the imidacloprid plot significantly lower numbers were found than in the teflubenzuron and emamectin benzoate treatments ($F_{4,8} = 5.33, p = 0.022$).

Table 5.5. Cumulative numbers (mean no. per trap \pm standard deviation) of the most abundant phytophagous insects and natural enemies, collected with two sampling methodologies, after the second insecticide application (sampling period from 24/09/2017 to 11/12/2017).

Taxa	Treatments					F value	p-value
	Teflubenzuron	Emamectin Benzoate	Imidacloprid	Cypermethrin	Control		
<u>Pitfall trapping</u>							
<i>N. simulans</i>	181.3 \pm 82.6	214.0 \pm 83.5	301.3 \pm 49.9	241.0 \pm 113.9	169.0 \pm 89.9	1.62	0.261
<i>Laminacauda</i> sp.	80.0 \pm 30.3	115.3 \pm 9.9	67.3 \pm 11.8	135.7 \pm 55.4	90.7 \pm 26.7	2.21	0.159
<i>B. peruvianus</i>	68.7 \pm 28.0	79.7 \pm 38.4	52.0 \pm 3.46	65.3 \pm 30.6	68.3 \pm 4.6	0.61	0.669
<i>Trimorus</i> sp.	33.7 \pm 16.3	33.3 \pm 7.5	19.7 \pm 4.7	35.7 \pm 17.9	28.0 \pm 10.6	0.78	0.568
<u>Pan traps</u>							
<i>N. simulans</i>	36.7 \pm 4.73	29.0 \pm 10.5	38.7 \pm 12.3	36.3 \pm 10.7	32.3 \pm 11.6	0.42	0.792
<i>F. occidentalis</i>	2634.0 \pm 188.9 ^a	2559.3 \pm 84.1 ^{ab}	2276.3 \pm 205.0 ^b	1892.0 \pm 54.7 ^c	2311.0 \pm 59.6 ^b	11.18	0.002
Dolichopodidae	100.0 \pm 38.2	179.7 \pm 121.3	124.3 \pm 34.7	53.3 \pm 42.3	138.7 \pm 125.7	1.14	0.403
Syrphidae	25.3 \pm 2.1	19.3 \pm 4.7	11.7 \pm 3.8	18.3 \pm 8.4	15.0 \pm 2.64	3.33	0.069
Aphidiinae*	33.3 \pm 13.0 ^{ab}	51.0 \pm 25.2 ^a	18.0 \pm 8.7 ^c	20.7 \pm 5.5 ^{bc}	27.0 \pm 7.0 ^{abc}	5.33	0.022

Different letters within a row indicate significant differences at $\alpha = 0.05$ (Duncan test), when the ANOVA was significant.

*ANOVA run after using Box–Cox transformation method $\gamma = -0.4$.

Table 5.6. Numbers of individuals of the most abundant insect natural enemies collected (mean no. per plant \pm standard deviation) under different treatments.

Taxa	Treatments					F value	p-value
	Teflubenzuron	Emamectin Benzoate	Imidacloprid	Cypermethrin	Control		
Aphidiinae wasps							
1DBA	1.66 \pm 1.53	1.67 \pm 0.88	2.22 \pm 1.35	1.44 \pm 0.84	1.00 \pm 0.67	0.96	0.481
<i>1st application</i>							
*6DAA	0.22 \pm 0.19	0.56 \pm 0.19	0.11 \pm 0.19	0.22 \pm 0.39	3.44 \pm 4.28	2.66	0.111
<i>2nd application</i>							
*6DAA	0.33 \pm 0.0	0.55 \pm 0.69	0.0 \pm 0.0	0.11 \pm 0.19	0.11 \pm 0.19	2.12	0.169
**69DAA	0.22 \pm 0.19 ^{ab}	0.11 \pm 0.19 ^b	0.0 \pm 0.0 ^b	0.22 \pm 0.19 ^{ab}	0.56 \pm 0.20 ^a	4.07	0.043
Predatory true bugs							
1DBA	0.0 \pm 0.0	0.2 \pm 0.17	0.23 \pm 0.40	0.33 \pm 0.35	0.0 \pm 0.0	N.A.	N.A.
<i>1st application</i>							
*6DAA	0.10 \pm 0.10	0.03 \pm 0.06	0.03 \pm 0.06	0.0 \pm 0.0	0.0 \pm 0.0	N.A.	N.A.
<i>2nd application</i>							
6DAA	0.22 \pm 0.39	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.22 \pm 0.19	N.A.	N.A.
69DAA	1.67 \pm 0.67 ^a	1.78 \pm 1.01 ^a	0.11 \pm 0.19 ^b	0.22 \pm 0.39 ^b	1.89 \pm 0.69 ^a	5.48	0.020
Syrphid larvae							
1DBA	0.0	0.0	0.0	0.0	0.0	N.A.	N.A.
<i>1st application</i>							
*6DAA	0.23 \pm 0.40	0.33 \pm 0.58	0.0 \pm 0.0	0.10 \pm 0.17	1.57 \pm 1.25	3.09	0.082
<i>2nd application</i>							
*6DAA	0.0 \pm 0.0 ^b	0.67 \pm 1.15 ^{ab}	0.0 \pm 0.0 ^b	0.0 \pm 0.0 ^b	0.56 \pm 0.19 ^a	5.39	0.021
1DBA	0.0	0.0	0.0	0.0	0.0	N.A.	N.A.
Chrysopid larvae							
1DBA	0.0	0.0	0.0	0.0	0.0	N.A.	N.A.
<i>2nd application</i>							
6DAA	0.0	0.0	0.0	0.0	0.0	N.A.	N.A.
69DAA	1.0 \pm 0.0 ^{ab}	0.53 \pm 0.69 ^{bc}	0.0 \pm 0.0 ^c	0.56 \pm 0.51 ^{bc}	1.56 \pm 0.51 ^a	4.35	0.037

DBA: days before application; DAA: days after application; NA: not applicable.

Different letters within a row indicate significant differences at $\alpha = 0.05$ (Duncan test), when the ANOVA was significant.

*ANOVA run after using Box–Cox transformation method $\gamma = -2.5$; ** $\gamma = 0.3$.

5.4. Discussion

The side effects of insecticides applied in staple crops (i.e., vegetables, legumes, rice, maize, citrus) and industrial crops (i.e., cotton, sugarcane, sugar beet) have been widely studied (Stanley & Preetha, 2016). Thus, relevant knowledge has been gained to improve integrated pest management schemes, taking the biological control services offered by a biodiverse agroecosystem into consideration (El-Wakeil et al., 2013). Hitherto, however, little is known about the unintentional effects of insecticides used in quinoa on non-target organisms (Bazile et al., 2014). The present field study provides information about the effects of four insecticides from different chemical groups (teflubenzuron, emamectin benzoate, imidacloprid and cypermethrin) on target and non-target arthropods in quinoa, assessed with three sampling methodologies: pitfall trapping for the ground dwelling arthropods, plant sampling for those that dwell on the quinoa plants and pan trapping for the insects that fly just above the crop canopy.

When an insecticide is incorporated into the cropping system, changes in the structure, richness and composition of the plant dwelling arthropod community may occur, which may eventually lead to a disruption of the ecosystem services provided by the beneficial fauna (Suttman & Barrett, 1979; Brown & Adler, 1989; Altieri, 1999; Letourneau & Goldstein, 2001). In the present study, foliar application with imidacloprid appeared to have a higher impact on arthropods residing in the quinoa crop than the other insecticides. For example, the richness in plant dwelling species was significantly lower in the imidacloprid treatment and also the species composition differed significantly from that in the other treatments and the untreated control.

Given that the soil surface of an agricultural system is in permanent interaction with the higher strata, changes in the plant dwelling arthropod community tend to precede changes in the structure of the ground dwelling species community (El-Naggar & Zidan, 2013). In this context, the reduction in species richness and the change in composition of species residing on the quinoa plants in the plots treated with imidacloprid may be related to the lower values of Shannon and Margalef indices found at the ground level as compared to the other treatments and the untreated control. The changes in the plant-associated community as a consequence of the insecticide application may have broken food webs that affected the incidence of a variety of species (Montoya et al., 2006; Tylianakis et al., 2007; Crowder & Jabbour, 2014). This may eventually be reflected in an altered species evenness and a reduction of species richness at the soil surface level, as observed in the present study (Youming et al., 2001). On the contrary, no differences between treatments and control were found in terms of species composition and

diversity of the insects collected in the yellow pan traps, probably due to the greater interaction at the top of the crop canopy with the areas surrounding the plots.

All of the tested insecticides substantially reduced densities of *S. recurvalis* larvae after the first application, which is in line with several previous studies that have demonstrated their efficacy against lepidopteran larvae (Ishaaya et al., 1986; Clarke-Harris et al., 2004; Shivankar et al., 2008; Manjula & Kotikal, 2018; Mead & Khedr, 2018; Muralikrishna et al., 2019; Jeschke et al., 2019). As reinfestation by *S. recurvalis* larvae did not occur in any of the plots, including the control, long-term effects of the insecticide treatments to control this pest could not be judged.

Whereas the efficacy of imidacloprid to control lepidopteran larvae by direct contact action has been demonstrated, this insecticide is also known to have an excellent systemic activity and therefore, the target organisms are mainly sucking insects such as thrips, aphids, whiteflies, leafhoppers and true bugs (Jeschke et al., 2019). Accordingly, the population densities of *M. euphorbiae*, *F. occidentalis* and *N. simulans*, which recurrently infested our plots, were significantly affected by the imidacloprid treatment.

The short-term effect of imidacloprid on *M. euphorbiae* was similar to that of the cypermethrin treatment. Differences between both treatments could be noted 69 days after the second application, with the imidacloprid plot having the lowest number of aphids per plant, likely due to its widely documented residual effects (Neuen, 1995; Devine et al., 1996; Elbert et al., 1998; El-Naggar & Zidan, 2013; Mohammed et al., 2018; Jeschke et al., 2019). On the other hand, teflubenzuron and emamectin benzoate had lesser effects on the aphids as compared to imidacloprid and cypermethrin; their impact on the aphid population as compared to the untreated control was noted 6 days after the first application. Teflubenzuron is reported to have low contact activity, but due to its systemic action in the plant it may cause toxicity to aphids by ingestion (Ishaaya & Degheele, 1998; Chakraborty & Chatterjee, 1999; Sánchez-Bayo et al., 2013; Jeschke et al., 2019). Contrarily, emamectin benzoate has no systemic activity but can kill the exposed aphids by direct contact (Jain et al., 2018; Jeschke et al., 2019).

Imidacloprid and cypermethrin had similar effects on *F. occidentalis* numbers, 6 days after both the first and second application. However, the residual effect of imidacloprid appeared to have prevented the infestation to a higher degree 69 days after the second application, resulting in the lowest number of thrips per plant. The effects of teflubenzuron and emamectin benzoate treatments on *F. occidentalis* were observed 6 days after the second application. On day 69 after the second treatment the residual activity of teflubenzuron may explain the significantly lower

mean number of thrips per plant (26.3 specimens) as compared to the emamectin benzoate treatment.

No visible short-term effects were observed on the population density of *N. simulans*, in any of the treatments, probably because the infestation was very low at the early stages of the crop when the insecticide treatments were done, and also because this pest was more abundant on the soil at this time. However, by its residual effect imidacloprid may have prevented a higher level of infestation by *N. simulans*, resulting in the lowest number of individuals per plant 69 days after the second application.

Given its broad-spectrum activity, imidacloprid may also affect non-target arthropods (Jeschke et al., 2019). These non-target organisms may be exposed to imidacloprid by direct contact, but the compound being highly systemic, non-target omnivorous insects (including natural enemies) that feed on plant fluids or pollen may also be exposed, even a relatively long time after an application (Pons & Albajes, 2001). Furthermore, reduction of prey densities (i.e., the target organisms) may eventually affect the beneficial fauna that will not be able to find sufficient food, facing a greater intra- and interspecific competition (Mills, 2006). In this context, with *M. euphorbiae* being highly affected by the imidacloprid treatment, there was a tendency towards lower numbers of individuals (even zero) of the aphidophagous guild on the plants as compared to the untreated control, both for the specialized natural enemies (such as the Aphidiinae wasps and predatory Syrphidae larvae) as for generalist predators (such as predaceous true bugs and chrysopid larvae). These observations are in line with previous studies indicating that imidacloprid affects key natural enemies of aphids such as coccinellids, anthocorids, geocorids, chrysopids, and Aphidiinae wasps (Prabhaker et al., 2011; Varenhorst & O'Neal, 2012; El-Naggar & Zidan, 2013; Roubos et al., 2014; Mohammed et al., 2018).

Due to its broad-spectrum activity, cypermethrin is considered to be harmful to a range of natural enemies by direct contact, but its relatively short residual activity suggests that a recolonisation of the crop by these organisms may occur sometime after an application (Jeschke et al., 2019). However, the observations in the present study indicate similar long-term effects of cypermethrin to those of imidacloprid on the different natural enemies collected. Teflubenzuron and emamectin benzoate sprays, however, tended to have less harmful effects on the beneficial fauna than the broad-spectrum insecticides used, which is in line with the literature (Ishaaya & Degheele, 1998; Jeschke et al., 2019); although environmental risks of emamectin benzoate such as toxicity to certain non-target arthropods and aquatic organisms have been reported (European Food Safety Authority, 2012).

One limitation of this study is that the field experiment was done over a single growing season only. To demonstrate the efficacy of an insecticide, it is recommended that trials be carried out in different locations or growing seasons (FAO, 2006). On the other hand, data provided about the side effects of the insecticides on the non-target species, particularly natural enemies, are in line with standard methods (Hassan, 1985), so this information is relevant for quinoa growers in order to take actions to improve their current use of the pesticides. Moreover, the data are in line with those of previous studies (Soca, 2021; Chapters 3 and 4) and the crop management practices are representative for quinoa cultivation in Peru and neighbouring countries (Bazile et al., 2014; Cruces et al., 2016; Gómez & Aguilar, 2016).

The results of this study indicate that due to the detrimental effects of imidacloprid on arthropod diversity, on the composition of species and specifically on the natural enemy population, foliar application of this active ingredient is less suitable for an IPM program in quinoa as compared to the other insecticides, in spite of its good performance in the control of the target pests. Teflubenzuron and emamectin benzoate substantially suppressed *S. recurvalis* larvae, with less negative effects than imidacloprid and cypermethrin to the beneficial fauna; however, further research is needed to evaluate the efficacy of both selective insecticides against quinoa pests in order to be considered as an element of an IPM package in quinoa. Due to the negative impact of cypermethrin on the natural enemy complex, restricted use is recommended for the management of quinoa pests.

Chapter 6

Thermal biology of *Liorhyssus hyalinus* (Fabricius) and *Nysius simulans* Stål, two hemipteran pests of quinoa

Redrafted after:

Cruces, L., de la Peña, E., and De Clercq, P. 2022. Thermal biology of *Liorhyssus hyalinus* (Hemiptera: Rhopalidae) and *Nysius simulans* (Hemiptera: Lygaeidae), fed on the milky stage of maize grains. *Journal of Insect Science* (accepted).

Author Contributions:

Conceptualization, L.C., E.d.I.P. and P.D.C.; methodology, L.C., E.d.I.P. and P.D.C.; formal analysis, L.C., E.d.I.P. and P.D.C.; investigation, L.C., E.d.I.P. and P.D.C.; data curation, L.C.; writing—original draft preparation, L.C.; writing—review and editing, L.C., E.d.I.P. and P.D.C.; supervision, E.d.I.P. and P.D.C.

6.1. Introduction

When quinoa is cultivated outside of its Andean origin, it can be severely infested by a broader range of phytophagous insects (Chapter 4). Two of these are the heteropteran pests *Liorhyssus hyalinus* (Fabricius) (Hemiptera: Rhopalidae) and *Nysius simulans* Stål (Hemiptera: Lygaeidae) which at the coastal level of Peru have been reported to cause serious problems in this crop (Cruces et al., 2016; Latorre, 2017). Both species have also been noted to be part of the quinoa pest complex in Argentina and Chile (Dughetti, 2015a; Chorbadjian et al., 2021).

L. hyalinus is a cosmopolitan species and in South America it has been reported from Argentina, Chile, Colombia, Ecuador, Paraguay, Peru and Venezuela (Göllner-Scheiding, 1976; Froeschner, 1981; Cermeli et al., 2004; Hradil et al., 2007; Prado, 2008; Chorbadjian et al., 2021). This bug has been recorded on a wide range of plants, both weeds and cultivated plants and in the latter, it can become an important pest (Wheeler, 2016). Adults of this rhopalid infest quinoa from the grain filling stage. Nymphs and adults suck water and nutrients from the developing grains, causing direct damage to quinoa production (Dughetti, 2015a; Gómez & Aguilar, 2016).

N. simulans is a neotropical species and has been noted to occur in Argentina, Brazil, Chile, Paraguay, Peru and Uruguay (Dalazen et al., 2014; Chorbadjian et al., 2021; Chapter 4). This soil dwelling species has a cryptic appearance and minute size and in consequence, it usually goes unnoticed by the farmer until population grows and starts climbing onto the weeds and crop plants where it can become an important pest. Adults of *N. simulans* infest quinoa during the grain filling stage, and both the nymphs and adults suck on the developing grains, causing economic damage (Dughetti, 2015a, 2015b; Gómez & Aguilar, 2016; Chapter 4).

Being poikilothermic organisms, temperature is a key environmental variable affecting development and reproduction of insects (Schowalter, 2016). Therefore, knowledge on the thermal biology of insect pests is essential not only to understand the life cycle of the species but also for pest risk analysis and integrated pest management (Sinclair et al. 2012; Mujica et al., 2017; Motswagole et al., 2019). The current study was undertaken to determine the effects of temperature on the developmental and reproductive parameters of *L. hyalinus* and *N. simulans* fed with fresh corn grains (*Zea mays* L.), an alternative host plant of these heteropterans (Dughetti, 2015b; Wheeler, 2016). The findings of the present study may be useful to predict their population dynamics of these heterometabolous insects in quinoa fields and make inferences on their potential distribution and peak densities throughout the year, according to the thermal conditions of the localities where quinoa is cultivated.

6.2. Materials and Methods

6.2.1. Stock culture

Colonies of *L. hyalinus* and *N. simulans* were established in December 2018 with nymphs and adults collected in the quinoa fields of the Cereal and Native Grains Program at the National Agrarian University La Molina, in Lima, Peru. Colonies of both species were established and maintained in the laboratories of the Museum of Entomology “Klaus Raven Büller” at ambient laboratory conditions (around 26-28 °C). The insects were housed in acrylic boxes of 20 x 20 x 20 cm with paper towelling on the bottom. The identity of *L. hyalinus* was confirmed with molecular tools: DNA extraction and PCR procedures were performed at the Department of Plants and Crops of Ghent University in Belgium (Chapter 5). *N. simulans* was identified by Dr. Pablo Dellapé from the Museo de La Plata in Argentina.

Adults and nymphs of both species were fed with fresh grains at milk stage of amylaceous corn which also served as a water source. For the adults of *N. simulans*, cotton rolls were provided as an oviposition substrate, where eggs were usually found individually or in small clusters of up to 10 eggs. For *L. hyalinus* no oviposition substrate was provided because eggs were laid on the corn grains and on the walls of the acrylic boxes, where they were usually found in clusters of around 10-20 eggs. Maintenance of the colony was done every 2-3 days during which grains were replaced by fresh ones, dead individuals were removed and, for the containers with adults, eggs were collected to start a new generation.

6.2.2. Experiments

Trials assessing developmental and reproductive parameters of both species were done in the laboratories of the Museum of Entomology “Klaus Raven Büller”, in a climatic cabinet (VISION SCIENTIFIC VS-3DM, South Korea) set at different constant temperatures (± 0.5 °C), $65 \pm 5\%$ RH, and a photoperiod of 14:10 (L:D) h.

For each species, adults from the stock culture were sexed, paired and transferred (at least 100 pairs) to Petri dishes (9 cm diameter, 1.5 cm high, lined with white cardboard) to the corresponding temperature at which the developmental performance of the offspring was to be assessed.

On the third day, eggs (< 1 hour old) were collected on the third day to be used in the development assays: for *N. simulans*, the cotton (oviposition substrate) was examined under a binocular stereoscope in order to collect the eggs (that remained stuck to the cotton strands) with fine forceps; for *L. hyalinus*, the eggs were collected under a binocular stereoscope, aided with a piece of paper to separate the eggs from the surface to which they were attached. Only

for the assay at 18 °C, eggs (< 1 hour old) collected directly from the stock colony were used to determine the egg and nymphal development, since the females transferred to 18 °C did not lay enough eggs for the experiment.

As in the stock culture, adults and nymphs in the different treatments were fed with fresh grains of corn which also served as a water source, and adults of *N. simulans* were provided with cotton rolls as oviposition substrate.

6.2.2.1. Egg and nymphal development

Egg and nymphal development were studied at six constant temperatures: 18, 22, 26, 30, 34 and 36 °C; except for the latter, these temperatures are in line with the yearly range of the daily maximum temperatures that may occur at the coastal areas of Peru (SENAMHI, 2021). In all treatments, nymphs were fed with fresh grains of corn, which were replaced with fresh ones depending on the temperature, i.e. daily at 30-36 °C or every other day at 18-26 °C.

For each treatment, the incubation time of the eggs was determined using 100-230 eggs (< 1 hour old). To facilitate the counting of hatched eggs, and to prevent egg cannibalism by hatchlings in *N. simulans*, the eggs were stuck on the adhesive side of a piece of masking tape, placed on a plastic Petri dish and kept at the studied temperature. As soon as the first egg hatched, the eggs were monitored every hour until the last egg hatched.

A second batch of eggs (<1 h old) was incubated at each temperature for monitoring nymphal development. From 80 to 140 first instars (1 day old) were individually caged in plastic Petri dishes (5 cm diameter, 1.3 cm high, lined with white cardboard) with a single fresh grain of corn. For practical reasons and in order to obtain accurate data of each instar duration, the nymphs were monitored at different time intervals according to the temperature, as follows: at the lowest temperatures (18 and 22 °C), every 24 h; at mid-range temperatures (26 and 30 °C) every 12 h and at the highest temperature (34 °C), every 8 h. At 36 °C, a preliminary assay indicated that nymphs were very susceptible to manipulation, resulting in mortality of 98.5% in *L. hyalinus* and 81.4% in *N. simulans*. To increase the nymphal survival and enable determining the total nymphal period of a representative number of nymphs, instars were not monitored at the latter temperature, and nymphs were taken out of the incubator every 24 h only to replace the food.

Newly emerged adults (< 12 h old) were sexed and weighed using a Mettler Toledo AL204 balance (Mettler-Toledo Group, China) and they were used in the assays to determine the reproductive parameters and longevity.

6.2.2.2. Reproduction

Adult reproduction was studied at 22, 26, 30 and 34 °C. Newly emerged adults (< 12 h old) coming from the nymphal development assays were paired and transferred to plastic Petri dishes (9 cm diameter, 1 cm high, lined with white cardboard) and then exposed to the same temperature and with the same food as in the nymphal period, but honeybee pollen was offered to the adults of *L. hyalinus* as a source of extra nutrients; preliminary observations indicated that *N. simulans* did not feed on the offered pollen. In all cases the minimum number of replicates (couples) was 11. Food (fresh corn and pollen grains) was replaced every other day.

Cotton rolls were provided as oviposition substrate to *N. simulans*, whereas the whole Petri dish could be used for oviposition by *L. hyalinus*. The cotton rolls or Petri dishes were daily checked until the first egg was laid to determine the pre-oviposition time. Thereafter, they were daily checked until the last egg was laid to calculate the oviposition period, but egg counts were done only every other day to determine total fecundity. Males were kept with their female mates until they died and longevity of both sexes was recorded.

In order to determine the egg viability (expressed by the percentage of egg hatching), all eggs laid by the monitored females at the different constant temperatures were stuck on the adhesive side of a piece of masking tape and placed on a plastic Petri dish, and then kept at the studied temperature until hatching.

6.2.3. Data analysis

All statistical analyses were performed using R software, version 3.4.2 (R Core Team, 2020), and all tests were analyzed at the significance level $\alpha = 0.05$.

For development and reproduction, differences between treatments were analyzed by using ANOVA tests, provided the data was/were normally distributed and homoscedastic as indicated by Shapiro Wilk and Bartlett tests, respectively. In case of heteroscedasticity, the Box Cox transformation method was used to stabilize the variances; however, untransformed data are presented in the tables. Means were separated using a Tukey test. When data was not normally distributed, the non-parametric Kruskal Wallis test was used to compare the treatments, followed by a Fisher's least significant difference test as a post hoc test.

Parameters expressed as percentages (proportion of ovipositing females and egg hatch) were compared by means of a logistic regression (family function = binomial) and groups were identified by the Tukey contrasts test. Means and SD-values were expressed as percentages. Calculations were performed in R, using the packages 'glm2' and 'multcomp' (Zhang & Rojas, 2010; Marschner & Donoghoe, 2018; Hothorn et al., 2022).

Sex ratios were evaluated versus an equal male:female distribution (1:1 ratio) by using a non-parametric Chi-square test.

The relation between temperature and development rate (1/development time) of eggs and nymphs was described by a linear regression model, which has been well documented to be suitable for estimation of lower development thresholds (LDTs) and thermal constants in several arthropods (Campbell et al., 1974; De Clercq & Degheele, 1992; He et al., 2003; Du Plessis et al., 2011; Bonte et al., 2012; Luybaert et al., 2014; Mujica et al., 2017). The equation fitted was " $Y = a + bX$ ", where Y is the development rate, X is the rearing temperature, and the regression parameters are the intercept (a), and the slope (b). The significance of the temperature in the fitted model was tested using a one-way ANOVA. The lower temperature thresholds of insect development were determined as the x-intercept ($t_o = -a/b$). For thermal requirements, the mean number of degree-days (DD) and standard deviations (from all individuals tested) were determined using the equation $DD = D(T - t_o)$ where D is the developmental time in days, T is the temperature (°C) during development and t_o is the lower developmental threshold (°C) (De Clercq & Degheele, 1992). LDTs and DD for the period from egg to preoviposition (as a single generation) were also calculated for both species. Thermal requirements of *L. hyalinus* and *N. simulans* were compared using a Mann Whitney test.

6.3. Results

6.3.1. Development

All nymphal instar durations and the total nymphal period of both species varied significantly with temperature, decreasing as the temperature increased up to 34 °C (for each monitored instar) or up to 36 °C (for the total nymphal development) (Table 6.1 and 6.2).

All instar durations could not be measured at the extreme temperatures of the tested range, i.e. at 18 °C due to high mortality observed in the assay (Table 6.1 and 6.2) and at 36 °C where a high mortality in the preliminary assays was noted.

At 18 °C, from the initial number of 140 first instars of *L. hyalinus* or *N. simulans*, not a single individual reached adulthood. For *L. hyalinus*, only 8.6% of the individuals reached the fifth instar, which eventually died within the following 12 days. For *N. simulans* only 17.1% of the individuals reached the second instar at 18 °C, which eventually all died; the remaining 82.9% of the first instar nymphs progressively died within 10 to 66 days after hatching. At 22 – 34 °C

nymphs of both species successfully reached adulthood, but with an apparent higher mortality in *L. hyalinus* than in *N. simulans* (Table 6.1 and 6.2).

Table 6.1. Duration in days (mean \pm SD) of the different instars and/or total nymphal period of *L. hyalinus* at six constant temperatures.

Temp (°C)	Instar ²						Total nymphal period ²
	N ¹	N1	N2	N3	N4	N5	
18	140	10.02 \pm 1.68a (43)	11.04 \pm 1.81a (27)	11.92 \pm 2.71a (25)	12.5 \pm 2.35a (12)	/ (0)	
22	125	5.52 \pm 0.72b (115)	3.78 \pm 0.43b (115)	3.40 \pm 0.51b (115)	3.88 \pm 0.53b (102)	6.25 \pm 1.21a (70)	22.77 \pm 2.04a (70)
26	80	4.23 \pm 0.90c (70)	3.12 \pm 1.00c (66)	3.18 \pm 1.49c (60)	3.11 \pm 0.59c (57)	4.90 \pm 0.88b (51)	18.27 \pm 1.97b (51)
30	112	2.16 \pm 0.21d (102)	1.45 \pm 0.10d (102)	1.47 \pm 0.12d (85)	1.62 \pm 0.12d (82)	2.67 \pm 0.30c (78)	9.35 \pm 0.38c (78)
34	96	1.28 \pm 0.07e (92)	1.23 \pm 0.18e (67)	1.21 \pm 0.17e (44)	1.29 \pm 0.14e (38)	2.28 \pm 0.13d (36)	7.27 \pm 0.39d (36)
36	110	/ (83)	/ (67)	/ (56)	/ (50)	/ (55)	6.08 \pm 0.51e (45)
χ^2		392.9	328.9	288.9	257.5	204.8	262.0
df		4	4	4	4	3	4

Different letters within a column indicate significant differences at $\alpha = 0.05$ (Kruskal Wallis test).

¹ Initial number of first instars tested.

² The number of surviving nymphs based on which the mean and SD values were calculated is placed in parentheses.

At 36 °C, instar periods (N1-N5) were not monitored.

Table 6.2. Duration in days (mean \pm SD) of the different instars and/or total nymphal period of *N. simulans* at six constant temperatures.

Temp (°C)	Instar ²						Total nymphal period ²
	N ¹	N1	N2	N3	N4	N5	
18	140	42.38 \pm 6.71a (24)	/ (0)	-	-	-	
22	82	16.93 \pm 3.89b (70)	11.34 \pm 2.45a (67)	9.41 \pm 1.35a (64)	9.36 \pm 0.99a (58)	12.25 \pm 1.19a (52)	59.85 \pm 7.13a (52)
26	98	9.37 \pm 1.76c (94)	6.93 \pm 1.38b (87)	6.35 \pm 1.17b (83)	6.34 \pm 0.94b (81)	8.01 \pm 0.81b (79)	36.82 \pm 4.03b (79)
30	88	4.72 \pm 0.70d (86)	3.14 \pm 0.47c (85)	2.97 \pm 0.38c (83)	3.13 \pm 0.41c (81)	4.53 \pm 0.40c (80)	18.42 \pm 1.59c (80)
34	101	2.36 \pm 0.22e (99)	2.25 \pm 0.29d (97)	2.10 \pm 0.19d (97)	2.17 \pm 0.23d (96)	3.44 \pm 0.37d (93)	12.35 \pm 0.78d (93)
36	87	/ (76)	/ (75)	/ (75)	/ (75)	/ (73)	12.29 \pm 0.96d (73)
χ^2		349.5	302.1	298.6	290.4	279.4	335.9
df		4	3	3	3	3	4

Different letters within a column indicate significant differences at $\alpha = 0.05$ (Kruskal Wallis test).

¹ Initial number of first instars tested.

² The number of surviving nymphs based on which the mean and SD values were calculated is placed in parentheses.

At 36 °C, instar periods (N1-N5) were not monitored.

The effects of temperature on nymphal survival (Table 6.3) were not compared statistically, because the nymphs were monitored at different time intervals in the different temperature treatments and therefore differences in the mortality rates among the treatments were probably also due to varying effects of manipulation. For instance, the nymphal survival of *L. hyalinus* at 36 °C was slightly higher than at 34 °C, probably because at the latter temperature the nymphs were examined (out of the climatic cabinet) every 8 hours, whereas at 36 °C the Petri dishes containing the nymphs were only taken out to replace the food every 24 hours.

Incubation times of eggs and total developmental times of nymphs of *L. hyalinus* and *N. simulans* at the tested temperatures are shown in Table 6.3 and 6.4, respectively. Developmental times significantly varied with temperature for eggs (*L. hyalinus*: $\chi^2_5 = 816.83$, $p < 0.001$; *N. simulans*: $\chi^2_5 = 890.44$, $p < 0.001$), male nymphs (*L. hyalinus*: $\chi^2_4 = 124.39$, $p < 0.001$; *N. simulans*: $\chi^2_4 = 171.96$, $p < 0.001$) and female nymphs (*L. hyalinus*: $\chi^2_4 = 136.46$, $p <$

0.001; *N. simulans*: $\chi^2_4 = 162.37, p < 0.001$), decreasing as the temperature increased from 18 to 36 °C for eggs and from 22 to 36 °C for nymphs.

The heaviest males and females of *L. hyalinus* emerged at 22 and 26 °C, whereas the lightest were observed at 34 and 36 °C (males: $\chi^2_4 = 50.67, p < 0.001$; females: $\chi^2_4 = 53.26, p < 0.001$) (Table 6.3). For *N. simulans* the heaviest males were observed at 22 and 30 °C, but females had similar weights at 22-34 °C; the lightest males and females emerged at the extreme temperature of 36 °C (males: $\chi^2_4 = 91.51, p < 0.001$; females: $\chi^2_4 = 73.19, p < 0.001$).

No significant deviations from a 1:1 ratio were found in the sex ratio of *L. hyalinus* at 22 °C ($\chi^2 = 1.43, p = 0.232$), 26 °C ($\chi^2 = 0.49, p = 0.484$), 30 °C ($\chi^2 = 0.82, p = 0.365$), 34 °C ($\chi^2 = 0.11, p = 0.739$) and 36 °C ($\chi^2 = 0.02, p = 0.881$). Likewise, proportions of males and females of *N. simulans* were similar at 22 °C ($\chi^2 = 0.08, p = 0.781$), 26 °C ($\chi^2 = 2.27, p = 0.132$), 30 °C ($\chi^2 = 0.50, p = 0.479$), 34 °C ($\chi^2 = 0.27, p = 0.604$) and 36 °C ($\chi^2 = 0.34, p = 0.558$).

Table 6.3. Developmental parameters (mean ± SD) of *L. hyalinus* at six constant temperatures.

Temp ^a (°C)	Nymphal ^b survival (%)	Egg incubation time (days) ^c	Nymphal period (days)		Adult weight (mg)		Sex ratio ^d (male:female)
			Male	Female	Male	Female	
18	0.0 ± 0.0 (140)	25.38 ± 0.99a (107)	/	/	/	/	/
22	56.00 ± 4.40 (125)	11.54 ± 0.26b (148)	22.68 ± 1.51a	22.84 ± 2.39a	9.03 ± 0.90a	12.09 ± 1.35a	1:1.33
26	63.75 ± 5.37 (80)	7.86 ± 0.34c (156)	17.75 ± 2.12b	19.22 ± 2.32b	9.54 ± 1.10ab	12.27 ± 1.59a	1:0.82
30	69.64 ± 4.34 (112)	4.99 ± 0.06d (130)	9.23 ± 0.29c	9.45 ± 0.41c	8.80 ± 0.51b	11.44 ± 0.65b	1:1.23
34	37.50 ± 4.94 (96)	3.74 ± 0.06e (165)	7.03 ± 0.33d	7.49 ± 0.28d	8.04 ± 1.03c	9.64 ± 2.00c	1:1.12
36	40.90 ± 4.69 (110)	3.51 ± 0.04f (132)	5.99 ± 0.45e	6.18 ± 0.57e	7.97 ± 0.91c	10.26 ± 1.37c	1:1.05

Different letters within a column indicate significant differences at $\alpha = 0.05$ (Kruskal Wallis test).

^a 69.3% of nymphs subjected to 18 °C died in the first instar, 11.4% in the second instar, 1.4% in the third instar, 9.3% in the fourth instar and 8.6% in the fifth instar.

^b The initial number of first instars tested is placed in parentheses.

^c The number of eggs tested is placed in parentheses.

^d Sex ratios did not differ significantly from a 1:1 ratio at $\alpha = 0.05$ (X^2 test).

Table 6.4. Developmental parameters (mean ± SD) of *N. simulans* at six constant temperatures.

Temp ^a (°C)	Nymphal ^b survival (%)	Egg incubation time (days) ^c	Nymphal period (days)		Adult weight (mg)		Sex ratio ^d (male:female)
			Male	Female	Male	Female	
18	0.0 ± 0.0 (140)	26.08 ± 0.92a (115)	/	/	/	/	/
22	63.41 ± 5.31 (82)	13.93 ± 0.26b (192)	58.92 ± 7.94a	60.70 ± 6.32a	1.64 ± 0.16a	2.39 ± 0.19a	1:1.08
26	80.61 ± 3.99 (98)	9.43 ± 0.49c (132)	36.77 ± 4.44b	36.93 ± 3.77b	1.49 ± 0.20b	2.39 ± 0.24a	1:0.75
30	90.91 ± 3.06 (88)	5.65 ± 0.12d (143)	18.36 ± 1.58c	18.44 ± 1.71c	1.56 ± 0.12a	2.41 ± 0.17a	1:0.86
34	92.08 ± 2.69 (101)	4.23 ± 0.15e (229)	12.36 ± 0.77d	12.34 ± 0.79d	1.40 ± 0.13c	2.33 ± 0.18a	1:1.11
36	83.91 ± 3.94 (87)	4.06 ± 0.13f (132)	12.32 ± 0.96d	12.26 ± 0.97d	1.22 ± 0.11d	2.02 ± 0.13b	1:1.15

Different letters within a column indicate significant differences at $\alpha = 0.05$ (Kruskal Wallis test).

^a 87.9% of nymphs subjected to 18 °C did not reach the second instar, the remaining 12.1% died in the second instar.

^b The initial number of first instars tested is placed in parentheses.

^c The number of eggs tested is placed in parentheses.

^d Sex ratios did not differ significantly from a 1:1 ratio at $\alpha = 0.05$ (X^2 test).

6.3.2. Day-degree model

The linear regression analysis of the relationship between temperature and development rate of the egg and nymphal stage indicated a good linear model fit both for *L. hyalinus* and *N. simulans* at the range of temperatures from 18 °C (eggs) or 22 °C (nymphs) to 36 °C (in all cases $R^2 > 93\%$ and $p < 0.001$) (Table 6.5, Figure. 6.1). The egg and nymphal development of *L. hyalinus* required 68.6 and 114.8 DD, respectively, and a lower development threshold of 16.0 °C for eggs and 17.9 °C for nymphs was estimated. For *N. simulans*, eggs and nymphs required 77.7 and 190.3 DD to complete development, respectively; the lower development threshold for eggs was 16.1 °C while for nymphs it was 19.7 °C. The thermal requirements and lower threshold temperature for one generation (egg-preoviposition) were 236.9 DD and 18.0 °C for *L. hyalinus* and 301.5 DD and 19.0 °C for *N. simulans*.

Degree day requirements for development of the egg and nymphal stages were significantly higher in *N. simulans* than in *L. hyalinus* ($p < 0.001$).

Table 6.5. Lower developmental thresholds (t_0), degree-day requirements (K) (means \pm SD) and linear regression equations with corresponding coefficients of determination (R^2) for the immature stages and for the egg-preoviposition period of *L. hyalinus* and *N. simulans* calculated for constant temperatures from 18 °C (eggs) or 22 °C (nymphs) to 36 °C.

Species	Stage	t_0 (°C)	K(DD)	Regression equation	R^2	F-value	p-value
<i>L. hyalinus</i>	Egg	16.0	68.6 \pm 8.0	Y = -0.2291 + 0.0143X	0.988	68350	< 0.001
	Nymph	17.9	114.8 \pm 20.2	Y = -0.1569 + 0.0089X	0.942	4500	< 0.001
	Egg-Nymph	17.5	181.7 \pm 24.2	Y = -0.0963 + 0.0055X	0.965	7686	< 0.001
	Egg-Pre oviposition	18.0	236.9 \pm 38.6	Y = -0.0759 + 0.0042X	0.956	3174	< 0.001
<i>N. simulans</i>	Egg	16.1	77.7 \pm 11.93	Y = -0.2037 + 0.0127X	0.9784	42560	< 0.001
	Nymph	19.7	190.3 \pm 33.4	Y = -0.1043+ 0.0053X	0.937	5549	< 0.001
	Egg-Nymph	19.3	260.5 \pm 38.4	Y = -0.0743+ 0.0039X	0.952	7413	< 0.001
	Egg-Pre oviposition	19.0	301.5 \pm 39.1	Y = -0.0634 + 0.0033X	0.958	4244	< 0.001

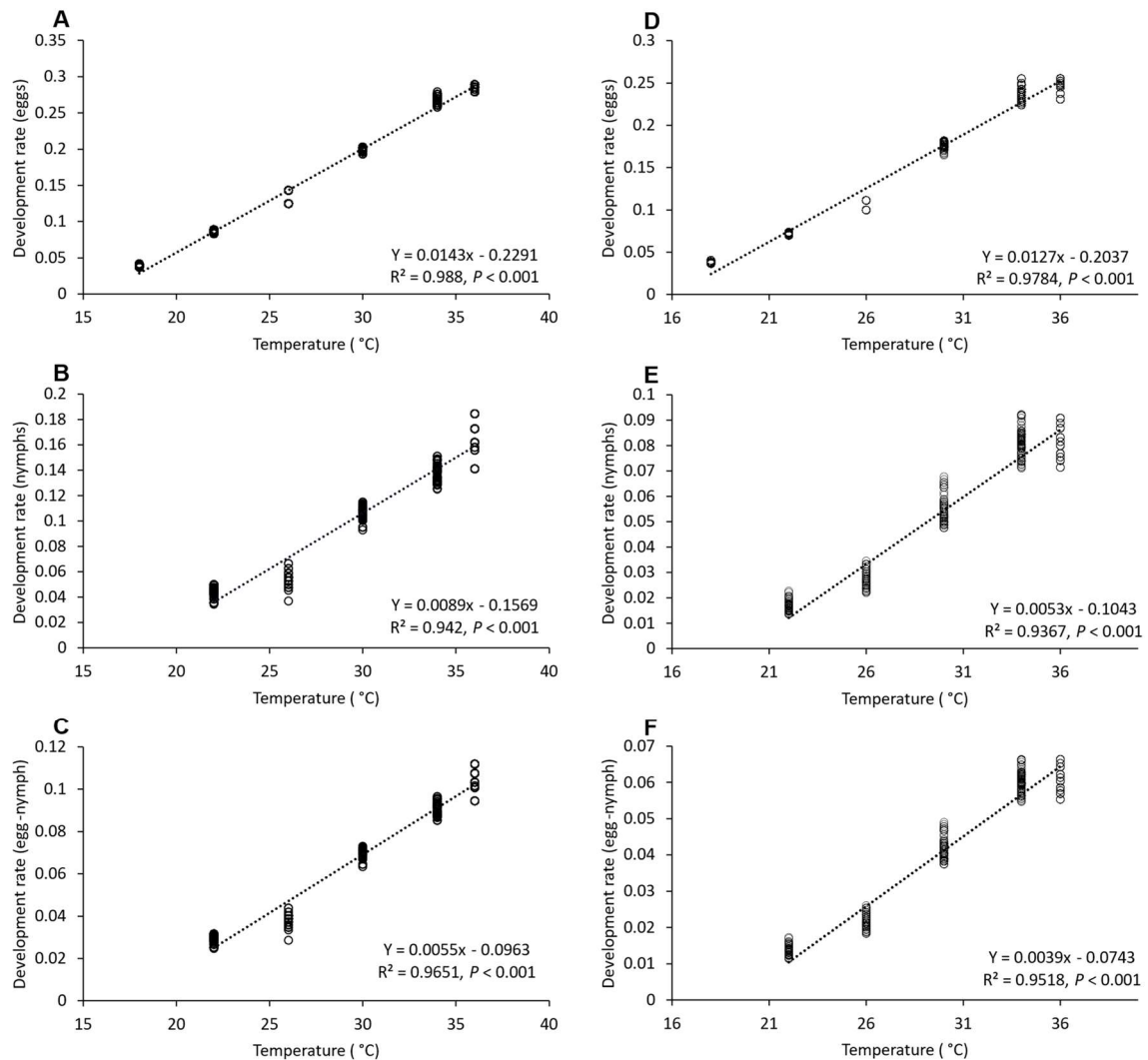


Figure 6.1. Linear relationship between temperature and developmental rate of egg, nymphal and egg-nymphal stages of *L. hyalinus* (A, B and C) and *N. simulans* (D, E and F). Broken lines represent linear regressions of all data from 18 °C (eggs) or 22 °C (nymphs) to 36 °C.

6.3.3. Reproduction and Longevity

No differences were found in the proportion of ovipositing females at 22, 26, 30 and 34 °C both for *L. hyalinus* ($\chi^2_3 = 3.98, p < 0.263$) and *N. simulans* ($\chi^2_3 = 1.31, p < 0.727$) (Table 6.6 and 6.7).

For *L. hyalinus* the preoviposition periods ranged from 3.6 to 13.8 days, and they significantly decreased as temperature increased, although there were no differences at the lower temperatures of 22 and 26 °C ($\chi^2_3 = 43.14, p < 0.001$). The oviposition periods ranged from 29.9 to 56.9 days, and were similar at 22, 26 and 30 °C, but significantly lower at 34 °C ($\chi^2_3 = 19.99, p < 0.001$) (Table 6.6).

Preoviposition times for *N. simulans* varied from 2.4 to 8.3 days, and they significantly decreased with increasing temperature ($\chi^2_3 = 61.03, p < 0.001$). The oviposition period was highest at 26 °C with 51.9 days on average, but there were no differences at 22, 30 and 34 °C ($F_3 = 14.1, p < 0.001$) (Table 6.7).

The lowest temperature (22 °C) significantly affected the fecundity both for *L. hyalinus* ($F_3 = 23.6, p < 0.001$) and *N. simulans* ($F_3 = 31.9, p < 0.001$). The highest fecundity was obtained at 30 °C with 553 eggs/female on average for *L. hyalinus* and 300 eggs for *N. simulans*; in the latter species, there were no differences in fecundity at 26 and 30 °C (Table 6.6 and 6.7).

Egg hatch during the total oviposition period was significantly affected by temperature regime. Egg hatch for both *L. hyalinus* ($\chi^2 = 1289.7, df = 3, p < 0.001$) and *N. simulans* ($\chi^2 = 5705.7, df = 3, p < 0.001$) was lowest at 22 °C: 30.1% and 15.9%, respectively. The highest egg hatch was observed at 30 °C: 95.9% for *N. simulans* and 67.1% for *L. hyalinus* (Tables 6.6, 6.7). For both heteropterans, the lowest tested temperature (22 °C) negatively affected the daily fecundity and egg hatch rates, while at 30 °C these rates reached higher values as compared with the other temperatures (Fig 6.2, Fig 6.3).

Adult longevity of *L. hyalinus* decreased as the temperature increased, being the longest at 22 and 26 °C and shortest at 30 and 34 °C, both for paired males ($F_3 = 21.76, p < 0.001$) ranging from 43.3 (34 °C) to 94.5 (22 °C) days on average, and for paired females ($F_3 = 24.30, p < 0.001$) ranging from 39.9 (34 °C) to 85.2 (26 °C) days on average (Table 6.6). The longest longevity of paired males of *N. simulans* was observed at 22 and 26 °C and the shortest at 30 and 34 °C ($F_3 = 18.9, p < 0.001$) ranging from 34.6 (34 °C) to 63.9 (26 °C) days on average; for paired females, the longest longevity was recorded at 26 °C and the shortest at 30 and 34 °C ($F_3 = 13.7, p < 0.001$), ranging from 33.9 (34 °C) to 58.3 (26 °C) days on average (Table 6.7).

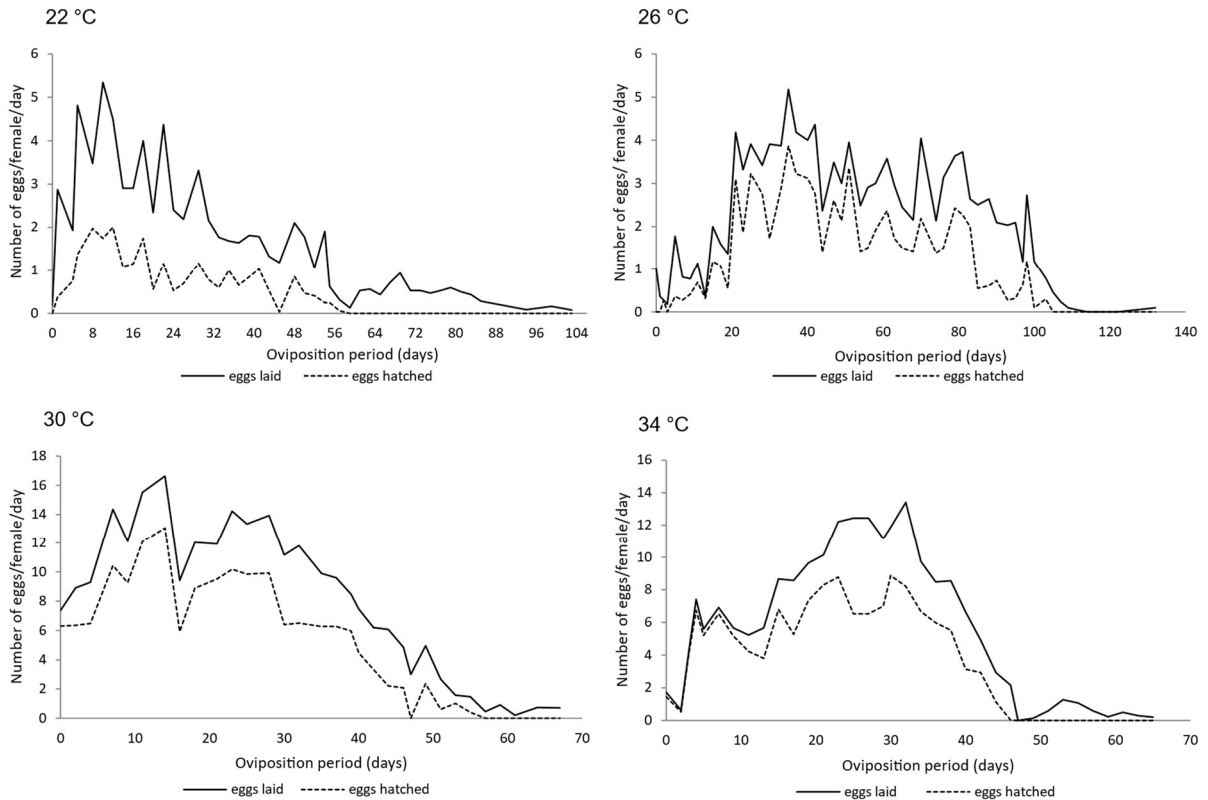


Figure 6.2. Daily mean fecundity and daily mean egg viability of *L. hyalinus* during its oviposition period at 22, 26, 30 and 34 °C.

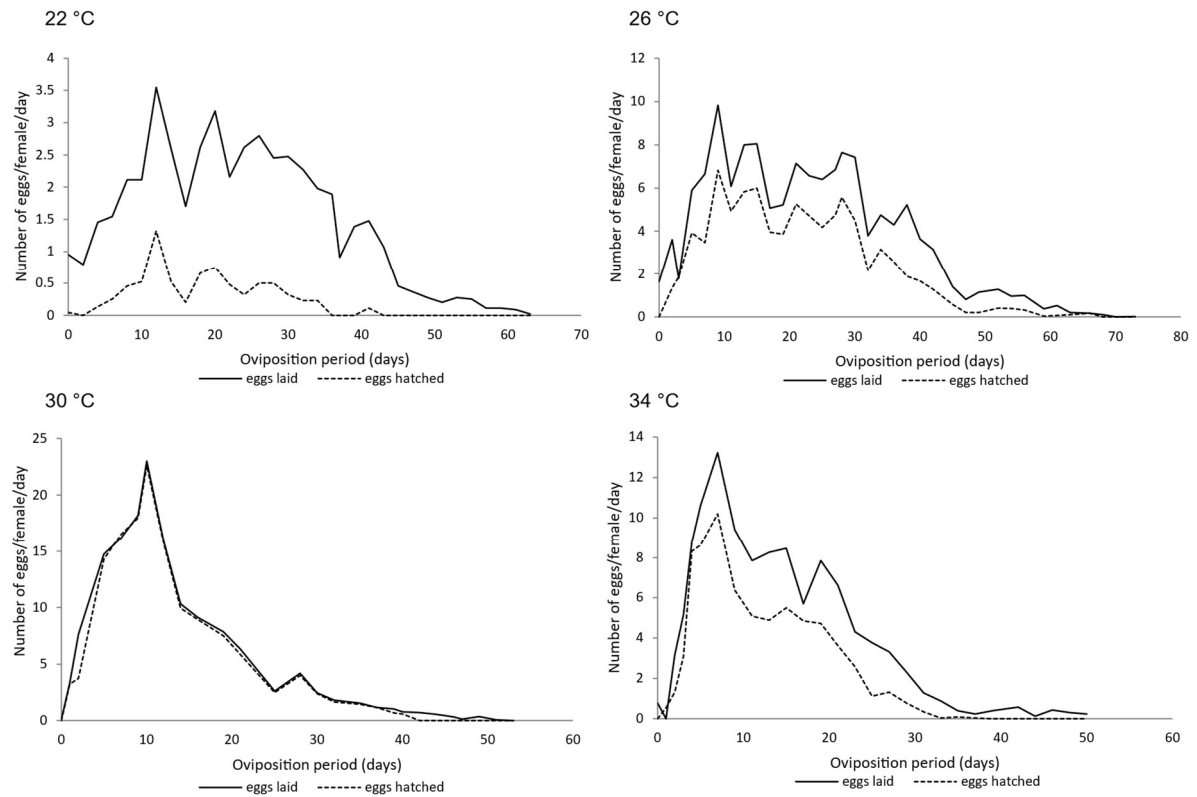


Figure 6.3. Daily mean fecundity and daily mean egg viability of *N. simulans* during its oviposition period at 22, 26, 30 and 34 °C.

Table 6.6. Reproductive parameters and longevities (means \pm SD) of *L. hyalinus* at four constant temperatures.

Temp (°C)	Proportion of ovipositing females ^a (%)	Pre-oviposition period (days)	Oviposition period (days)	Fecundity ^b (eggs/female)	Egg Hatch (%) ^b	Longevity (days)	
						Male ^c	Female ^c
22	88.9 \pm 0.1a (18)	13.8 \pm 4.0a	56.9 \pm 28.8a	169.5 \pm 119.3c	30.1 \pm 0.9c	94.5 \pm 27.7a	79.3 \pm 19.9a
26	100.0 \pm 0.0a (11)	11.8 \pm 3.1a	68.2 \pm 31.2a	275.4 \pm 121.6bc	59.7 \pm 0.9b	88.9 \pm 26.1a	85.2 \pm 25.1a
30	100.0 \pm 0.0a (17)	6.3 \pm 2.5b	44.0 \pm 9.9a	552.8 \pm 158.3a	67.1 \pm 0.5a	53.8 \pm 19.4b	51.7 \pm 13.8b
34	93.8 \pm 0.1a (16)	3.6 \pm 1.1c	29.9 \pm 9.4b	384.7 \pm 132.7b	66.6 \pm 0.6a	43.3 \pm 10.8b	39.9 \pm 8.7b

Different letters within a column indicate significant differences at $\alpha = 0.05$: Tukey contrast test (ovipositing females and egg hatch), Kruskal Wallis test (pre-oviposition and oviposition period), Tukey test (fecundity, adult longevity).

^a The number of adult pairs tested at each temperature is placed in parentheses.

^b Based on the total number of eggs laid per treatment.

^c ANOVA run after using Box-Cox transformation, $\lambda = 0.5$.

Table 6.7. Reproductive parameters and longevities (means \pm SD) of *N. simulans* at four constant temperatures.

Temp (°C)	Proportion of ovipositing females ^a (%)	Pre-oviposition period (days)	Oviposition period ^b (days)	Fecundity (eggs/female)	Egg Hatch (%) ^c	Longevity (days)	
						Male ^d	Female ^e
22	95.7 \pm 4.3a (23)	8.3 \pm 1.6a	29.1 \pm 8.6b	94.6 \pm 56.6c	15.85 \pm 0.8c	58.9 \pm 15.9a	45.2 \pm 11.5b
26	93.3 \pm 6.4a (15)	5.3 \pm 1.0b	51.9 \pm 15.8a	265.6 \pm 95.1ab	62.59 \pm 0.8b	63.9 \pm 25.0a	58.3 \pm 15.9a
30	95.7 \pm 4.3a (23)	2.8 \pm 0.5c	27.6 \pm 10.1b	299.5 \pm 79.2a	95.91 \pm 0.2a	34.9 \pm 10.6b	34.7 \pm 11.9c
34	100.0 \pm 0.0a (13)	2.4 \pm 0.2d	27.9 \pm 8.1b	200.8 \pm 59.2b	62.18 \pm 0.9b	34.6 \pm 10.7b	33.9 \pm 6.7c

Different letters within a column indicate significant differences at $\alpha = 0.05$: Tukey contrast test (ovipositing females and egg hatch), Kruskal Wallis test (pre-oviposition period), Tukey test (oviposition period, fecundity, adult longevity).

^a The number of adult pairs tested at each temperature is placed in parentheses.

^b ANOVA run after using Box-Cox transformation, $\lambda = 0.1$.

^c Based on the total number of eggs laid per treatment.

^d ANOVA run after using Box-Cox transformation, $\lambda = -0.3$, ^e $\lambda = -0.1$.

6.4. Discussion

Few studies have addressed the biology of *L. hyalinus* and life history data provided in the literature are scattered, poorly described or are in the grey literature (Readio, 1928; Hradil et al., 2007; Cornelis et al., 2012; Ríos, 2014; Arenas, 2019). Similarly, little is known on the biology of *N. simulans* and much of the work is unpublished (Mamani, 2015; Vásquez, 2016; Maquera, 2018). In this context, the present study provides more detailed information on the developmental and reproductive biology of both hemipteran pests.

Developmental rates of the eggs and nymphal instars of *L. hyalinus* and *N. simulans* increased significantly with temperature from 18 °C (eggs) or 22 °C (nymphs) to 36 °C. At the lowest tested temperature (18 °C) there was a pronounced prolongation of the egg incubation time, but the nymphs that emerged at this temperature did not succeed in reaching adulthood; however, the fact that 8.6% of the nymphs of *L. hyalinus* reached the fifth instar and that 82.9% of the *N. simulans* first instars stayed alive within a range of 10 to 66 days after hatching, may suggest that the nymphs can tolerate 18 °C for some period of time. Since eggs used in the assay at 18 °C were those that had been laid by females from the stock colony at a higher temperature (26-28 °C), these results can be partly influenced by maternal effects (Mousseau & Dingle, 1991; Gilchrist & Huey, 2001). The calculated lower developmental threshold temperature for nymphs of *L. hyalinus* was 17.9 °C, slightly lower than the lowest tested temperature (18 °C). For this reason, some development was observed in the nymphal stage. Contrarily, the LDT for nymphs of *N. simulans* was 19.7 °C, much higher than the lowest tested temperature; this may explain why almost no nymphal development was observed, and most of the nymphs die in the first instar.

Significant differences of nymphal periods of *L. hyalinus* were found between the highest tested temperatures (34 and 36 °C), and the tendency of the developmental rate shown Figure 6.1 B suggests that the maximum developmental rate has not been reached. For *N. simulans*, no differences of nymphal development were found between the highest tested temperatures (34 and 36 °C), and the tendency of the developmental rate shown in Figure 6.1 E suggests that the maximum developmental rate has been reached. These temperatures, however, do not coincide with the prevalent temperatures during the growing season of quinoa in Peru.

Results from previous studies in grey literature on the development of *L. hyalinus* and *N. simulans* are difficult to compare with our findings because they were carried out under ambient laboratory conditions, with varying temperatures and relative humidity yielding non-replicable results (Mamani, 2015; Vásquez, 2016; Maquera, 2018; Arenas, 2019). Ríos (2014) studied the

biology of *L. hyalinus* fed with quinoa at 21.8 ± 1.3 °C and $48.3 \pm 8.3\%$ RH. The latter author registered mean egg incubation and nymphal periods of 13.95 and 28.11 days, similar to our findings at 22 °C. Atalay (1978, cited in Hradil et al. (2007)) reported that eggs hatched after 5 days at 25 °C and after 3 days at 34 °C; the development of the first four instars (N1-N4) took on average 2 days each at 25 °C and 1 day at 34 °C, whereas for N5 it took 3 days at 25 °C and 2 days at 34 °C; these results differ substantially from our findings.

Sex ratios both in *L. hyalinus* and *N. simulans* were essentially 1:1 at the different constant temperatures, suggesting that males or females do not have a selective survival advantage as a function of temperature. Similar ratios were observed in previous studies carried out at temperatures within the range of the current study, for *L. hyalinus* (Ríos, 2014; Arenas, 2019) and *N. simulans* (Hradil et al., 2007; Mamani, 2015; Vásquez, 2016; Maquera, 2018). A sex ratio of 1:1 was also found in a related species, *Nysius huttoni* White, at different constant temperatures (He et al., 2003).

Non-linear models may enable a more accurate description of the relationship between temperature and developmental rate of an insect, as compared with linear models. However, linear regression (as used in the current study) permits calculations of thermal constants (DD) which can be of practical value for predicting population development in the field. This widely used parameter for IPM purposes cannot be generated by such non-linear models (Mujica et al., 2017; Kontodimas et al., 2004).

The thermal requirements and the estimated lower threshold temperature for the egg-nymphal development of *N. simulans* (260.5 DD and 19.3 °C) were higher than those of *L. hyalinus* (181.7 DD and 17.5 °C). This suggests that *L. hyalinus* may develop at lower temperatures than *N. simulans* and may complete more generations through the year. Such LDTs are relatively higher than those reported for other species, and only in few species the LDTs were found to be between 17 and 20 °C (e.g. *Liposcelis paeta* Pearman, *Tribolium castaneum* (Herbst) and *Latheticus oryzae* Waterhouse) (Stejskal et al., 2019). However, there are various factors that may affect the LDTs, including the geographical distribution of the species and specific thermal adaptations of local strains, which may result in substantial intraspecific variation in LDT values (Stejskal et al., 2019).

The lower developmental threshold calculated for *L. hyalinus* (17.5 °C) is in line with the findings of Atalay (1978) (i.e. 17.2 °C), but this author mentioned a thermal constant (218.4 DD) much higher than that found in the present study (181.7 DD). There has been no other previous

attempt to determine the thermal requirements and lower thresholds for *L. hyalinus* nor for *N. simulans*.

The thermal biology has been documented for other *Nysius* species that are agricultural pests, including *N. huttoni*, *Nysius vinitor* Bergroth and *Nysius ericae* Schilling (Slater, 1964; Kehat & Wyndham, 1972; He et al., 2003; Du Plessis et al., 2011). In all of these studies, the *Nysius* spp. were fed on sunflower seeds. The estimated mean low thresholds for egg development reported for *N. huttoni*, *N. vinitor* and *N. ericae* were 11.5, 14.5 and 13.9 °C, respectively; while for the nymphal development threshold temperatures were noted to be 10, 15 and 15.2 °C, respectively. These values are substantially lower than our results, with lower thresholds of 16.1 °C for the eggs and 19.9 °C for the nymphs. The degree-day requirements of *N. simulans* to complete its egg and nymphal development averaged 77.1 and 188.0 in the present study, which are similar to those obtained for *N. ericae* (74.6 and 200.0 DD) and *N. vinitor* (70.0 and 225.0 DD) but very different from those in *N. huttoni* (96.9 and 588.0 DD). These differences may be in part attributed to the geographical distribution of the (sub-)tropical vs temperate species: *N. huttoni* is native to New Zealand with a mean annual temperature ranging from 10 to 16 °C (Mullan et al., 2006).

Temperature did not have a significant effect on the proportion of ovipositing females, but it did have on the other reproductive parameters. Lower temperatures had a negative impact on oviposition: when females of either species from the stock colony (26 - 28 °C) were transferred to an incubator at 18 °C to collect their eggs, the oviposition rate notoriously decreased; in both species, the lowest fecundity and egg hatch were observed at 22 °C. A trend towards lower fecundity of *L. hyalinus* as temperature decreased was also recorded by Atalay (1978). In the present study, the most optimal temperature for both species was 30 °C, at which the highest fecundity and egg viability were observed. The maximum fecundity of *L. hyalinus* was on average 553 eggs/female, similar to the observations of Readio (1928) in summer conditions with temperatures at midday above 30 °C (i.e. 558 eggs). The maximum fecundity of *N. simulans* at 26 and 30 °C averaged 266 and 300 eggs/female, respectively, which is in line with the values reported for *N. ericae* at 26 and 28 °C (246 and 276 eggs/female, respectively), but considerably lower than those reported for *N. vinitor* at 25 and 30 °C (578 and 542 eggs/female, respectively). For *N. huttoni*, a very low fecundity was registered at 20 and 25 °C (12.93 and 11 eggs/female, respectively).

Our results indicate that both heteropterans are not well adapted to the temperatures of 18 and 22 °C: the lowest fecundity was observed at 22 °C and at 18 °C the nymphs could not reach

adulthood. This may explain why both species have not been recorded in the highlands of Peru characterized by its relatively low temperatures; for example, in Jauja (over 3,000 m a.s.l.) the annual maximum temperature averages 19.5 ± 2.2 °C, whereas the minimum temperature averages 4.5 ± 3.4 °C (data from 2008 to 2012, (SENAMHI, 2021)). At lower elevations (i.e., Majes, 1,410 m a.s.l.; La Molina, 343 m a.s.l.; Olmos, 175 m a.s.l.), however, where these heteropterans have been recorded causing damage on crops, the annual maximum temperatures are around 25 °C (data from 2009 to 2013, (SENAMHI, 2021)), which constitute better conditions for these species (Cruces et al., 2016; Gómez & Aguilar, 2016; Latorre, 2017; Soca, 2021; Chapter 4).

Data of reproductive parameters suggests that 30 °C is optimal for both species; this temperature is usually reached in summer (January to March) in the coastal region (SENAMHI, 2021). Considering that quinoa in the coastal region of Peru is usually sown in winter (i.e., between June to August), in late sowings the crop maturation and harvest coincide with the summer (in January) when peak numbers of *L. hyalinus* and *N. simulans* are present (Gómez & Aguilar, 2016). Recent studies have yielded suitable varieties of quinoa adapted to warm conditions for spring-summer sowings; however, the promising varieties will eventually be faced with the phytosanitary problems posed by these bugs when cultivated on a larger scale (Villena, 2011; Marca, 2015; Antezana-Febres et al., 2019).

Taking as a reference the meteorological data of Lima, where the daily mean temperature during the year ranges approximately from 17.5 to 25.5 °C (averaging 21.5 °C), and based on the DD requirements for egg to pre-oviposition (236.9 DD), an average of 5.3 generations of *L. hyalinus* and 3.0 generations of *N. simulans* can theoretically be expected in a year, meaning that both species are multivoltine.

Since the studied heteropterans were assessed with amylaceous corn grains as food, due to the difficulty of obtaining quinoa grains of the same stage (milky grain) during a long period of time (to carry out all experiments), further research using quinoa grains as food is needed. Both types of grains differ in their nutritional content (see Annex 1), and this may be reflected in differences in the performance of both heteropterans as a function of temperature. However, the current study does contribute to a better understanding of the geographical distribution of *L. hyalinus* and *N. simulans* in Peru based on the temperature regimes characterizing the regions where quinoa has been cultivated. The information gathered may also be useful from an agronomic point of view to improve the management of quinoa. For instance, it may assist in settling proper sowing times of quinoa, avoiding late sowings in order to prevent the coincidence of grain

maturation with periods in the summer when peak pest numbers are expected. Including a fallow period or practicing a crop rotation system during the summer may be a useful strategy to prevent damage by both heteropteran pests. Our results may be also useful as a starting point to lead further studies determining the lethal temperatures of both heteropterans and their survival mechanisms at unfavourable temperatures.

Chapter 7

Development, predation and prey preference of *Chrysoperla externa* on *Liorhyssus hyalinus* and *Nysius simulans*, two emerging pests of quinoa

Redrafted after:

Cruces, L., de la Peña, E., Livia, C. and De Clecq, Patrick. 2022. Development, predation and prey preference of *Chrysoperla externa* on *Liorhyssus hyalinus* and *Nysius simulans*, two emerging pests of quinoa. *Neotropical Entomology* (accepted)

Author Contributions:

Conceptualization, L.C., E.d.I.P. and P.D.C.; methodology, L.C., E.d.I.P., C.L. and P.D.C.; formal analysis, L.C., E.d.I.P. and P.D.C.; investigation, L.C., E.d.I.P. and P.D.C.; data curation, L.C.; writing—original draft preparation, L.C.; writing—review and editing, L.C., E.d.I.P. and P.D.C.; supervision, E.d.I.P. and P.D.C.

7.1. Introduction

Over the last decade, quinoa (*Chenopodium quinoa* Willd.) has been increasingly cultivated outside of its Andean origin, not only in South America but also around the world (Alandia et al., 2020). Since the crop was introduced at lower elevations, several insect pests have emerged causing losses to the quinoa fields (Latorre, 2017; Chapter 4). Two of these pests in South America are the heteropterans *Liorhyssus hyalinus* (Fabricius) (Hemiptera: Rhopalidae) and *Nysius simulans* Stål (Hemiptera: Lygaeidae), which were not part of the known pest complex of quinoa in the traditional cultivation areas (Saravia et al., 2014). These true bugs have been reported to cause serious problems in quinoa grown at the coastal level to about 1,400 m a.s.l., in Peru (Cruces et al., 2016; Gómez & Aguilar, 2016; Latorre, 2017). In Argentina and Chile, both species have also been documented to be part of the quinoa pest complex (Dughetti, 2015a, 2015b; Chorbadian et al., 2021).

Both species, *L. hyalinus* and *N. simulans*, may infest quinoa fields at high densities, mainly from the grain filling to maturation stage, when these true bugs climb to the panicle to suck the photosynthates of seeds, causing direct damage by reducing weight grains (Dughetti, 2015a, 2015b). At this crop stage, the application of insecticides involves the risk of harvesting grains with residues, which may eventually be rejected in the market due to the potential health hazard (Bedoya-Perales et al., 2018a). Under this context, a biological control method can be a more appropriate option that needs to be explored (Baker et al., 2020).

Among the predatory species that are part of the natural enemy guild in quinoa agroecosystems are the green lacewings (Neuroptera: Chrysopidae) (Valoy et al., 2015; Chapter 4). Lacewing larvae are voracious generalist predators, feeding on a wide range of insects. Prey of lacewings may include species of Sternorrhyncha (aphids, whiteflies, psyllids, mealybugs, scale insects), Auchenorrhyncha (leafhoppers), Thysanoptera, Lepidoptera (eggs and small larvae) and Acari (Canard, 2007). However, heteropterans as lacewing prey are barely studied, with only a few cases provided in literature (e.g. the plant bug *Creontiades pallidus* Rambur) and the lace bug *Stephanitis pyrioides* (Scott)) (Jafari et al., 2006; Rinehart & Boyd, 2006).

Green lacewings have been widely studied and successfully used in biological control programs (Senior & McEwen, 1998; Souza et al., 2019; Venzon et al., 2021). One of the most important species with a neotropical distribution is *Chrysoperla externa* (Hagen) (Albuquerque et al., 2007; Gamboa et al., 2016). This lacewing species is very common in the coastal areas of Peru and has been collected in quinoa fields (Sánchez & Vergara, 2005; Cruces et al., 2016). Moreover, it has been deemed as an excellent potential biological control agent because of its ability to adapt to

different cropping ecosystems where they prey on a range of economically important pests (Albuquerque et al., 1994; Carvalho & Souza, 2000; Garzón et al., 2015; Gamboa et al., 2016).

In the present study, we assessed *C. externa* as a predator of *L. hyalinus* and *N. simulans* by determining its developmental and predation rates when feeding on the immature stages of both pest species. Furthermore, in the field the predator may be diverted by the presence of other potential prey; thus, prey preference was studied using *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae), a polyphagous aphid that regularly infests quinoa fields, as an alternate prey (Chapter 4).

7.2. Materials and Methods

7.2.1. Insect cultures

Colonies of the predator *C. externa* and the hemipteran prey *L. hyalinus* and *N. simulans* were established and maintained in the laboratories of the Museum of Entomology “Klaus Raven Büller” at the National Agrarian University La Molina, at ambient laboratory conditions at around 26-28 °C. The aphid *M. euphorbiae* was reared at around 20-22 °C.

7.2.1.1. *C. externa*

Eggs of *C. externa* were obtained from the Peruvian National Service for Plant and Animal Health (SENASA) in Lima, Peru. The eggs were placed in plastic containers of 35 x 24 x 11 cm (Length x Height x Width) lined with paper towels, and supplied with folded cardboard to reduce larval cannibalism. The top was covered with a piece of tulle and with a perforated lid to provide ventilation. The eclosed larvae were fed every other day with frozen eggs of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), which was also obtained from SENASA.

When larvae showed signals of pre-pupation behaviour, pieces of corrugated cardboard were placed inside the rearing containers in order to provide a hiding site for pupation. Pupae were removed after 5-6 days and then transferred to an acrylic box of 40 x 30 x 30 cm (LxWxH) lined with paper towels and provided with a piece of kraft paper as oviposition substrate on top, covered with a perforated lid to provide ventilation. The emerging adults were fed with a mixture of honey, bee pollen, yeast, and water (6:0.25:10:15), which was placed over plastic strips (20 cm long, 3 cm width) located inside and reclined on the wall of the acrylic box. Water was provided via a piece of moistened cotton pad. The kraft paper containing the eggs was replaced daily or every 12 h (for the experiments).

Eggs of < 12 h old were used for the predation experiments. They were placed in Petri dishes (9 cm diameter, 1.5 cm high, lined with white cardboard) and kept in a climatic cabinet at 26 ± 0.5 °C, $65 \pm 5\%$ RH, and a photoperiod of 14:10 (L:D) h to complete the egg incubation period. Emerging larvae were used directly or reared on to the 2nd or 3rd instar, depending on the experiment, using *S. cerealella* eggs as food. First and/or second generation of the lacewings obtained in the laboratory were used in the experiments.

7.2.1.2. *L. hyalinus* and *N. simulans*

Colonies of *L. hyalinus* and *N. simulans* were established in December 2018 with nymphs and adults collected in the quinoa fields of the Cereal and Native Grains Program at the National Agrarian University La Molina, in Lima, Peru. The insects were housed in acrylic boxes of 20 x 20 x 20 cm (LxWxH) with paper towelling on the bottom. The identification of *L. hyalinus* was confirmed based on DNA extraction and PCR procedures performed at the Department of Plants and Crops of Ghent University in Belgium (Chapter 5). *N. simulans* was identified by Dr. Pablo Dellapé from the Museo de La Plata in Argentina.

As described in Chapter 6, adults and nymphs of both species were fed with fresh grains at milk stage of amylaceous corn (*Zea mays*) which also served as a water source. For the adults of *N. simulans*, cotton rolls were provided as an oviposition substrate. For *L. hyalinus* no oviposition substrate was provided because eggs were laid on the corn grains and on the walls of the acrylic boxes. Maintenance of the colony was done every 2-3 days, replacing sucked grains by fresh ones, removing dead individuals and, for the containers with adults, collecting eggs to start a new generation, or to be used in the predation experiments.

Eggs collected for the experiments were less than 24 h old and were placed in Petri dishes (9 cm diameter, 1.5 cm high, lined with white cardboard) to complete the incubation period and different nymphal stadia (N1-N5) depending on the experiment.

7.2.1.3. *M. euphorbiae*

Colonies of the aphids were established in October 2021 with nymphs and adults collected in the quinoa fields of the Cereal and Native Grains Program at the National Agrarian University La Molina, in Lima, Peru. The identity of *M. euphorbiae* was confirmed with molecular tools: DNA extraction and PCR procedures were performed at the Department of Plants and Crops of Ghent University in Belgium (Chapter 5). The identity of the collected specimens was confirmed by taxonomic keys provided in the literature (Blackman & Eastop, 2000, 2006).

For rearing the aphids, a modified method of Sidney et al. (2010) was used as follows: pieces of infested quinoa plants were placed in Petri dishes (9 cm diameter, 1.5 cm high), with a piece of

butterhead lettuce (*Lactuca sativa* var. *capitata* L.), specifically the midrib, where the aphids moved to when the quinoa tissues started to dry out. Pieces of lettuce were replaced every other day at which time dead or parasitized aphids were removed.

The new healthy aphid colonies were transferred to a circular plastic container (11 cm diameter, 5 cm high) with paper towelling on the bottom and covered with tulle and a holed lid at the top, containing three pieces of lettuce midribs lined in the perimeter of the container. Every three days (when lettuce midrib showed signals of dehydration), adult females were moved to new piece of lettuce with the aid of a paint brush to start a new rearing container. For the experiments, aphids of 2 days old (i.e., late first to early second instar nymphs) were used, which belonged to the second or third generation of rearing.

7.2.2. Experimental set up

All experiments were done in the laboratories of the Museum of Entomology “Klaus Raven Büller”, in a climatic cabinet (VISION SCIENTIFIC VS-3DM, South Korea) set at a constant temperature of 26 ± 0.5 °C, $65 \pm 5\%$ RH, and a photoperiod of 14:10 (L:D) h.

7.2.2.1. Predation rates

Predation rates of *C. externa* instars were assessed using the immature stages (eggs and all nymphal instars) of *L. hyalinus* and *N. simulans* as prey (Table 7.1).

Preliminary assays with 5-10 replicates of every predator/prey combination determined the number of prey needed for offering *ad libitum* to each chrysopid instar (Table 7.1). In these trials, newly emerged or moulted (<24 h) lacewing larvae were caged in individual plastic Petri dishes (5 cm diameter, 1.3 cm high, lined with white cardboard) and kept for 24 h of starvation; thereafter, they were offered the prey *ad libitum* during 24 h, after which the prey consumption was quantified.

The number of prey items needed for each predator-prey combination in the final experiment (Table 7.1) were transferred with a fine brush to 5-cm Petri Dishes (as above) containing a single fresh grain of corn (as a source of water and food for the prey). The Petri dishes were transferred to the climatic cabinet and after another 12 h, any nymphs that died as a result of manipulation were replaced by healthy ones.

Newly emerged first instar larvae of *C. externa* were individually housed in 5-cm Petri dishes and initially fed with *S. cerealella* eggs. After the first six to 12 h within each tested instar, larvae were starved for 24 h without access to water. After starvation, each larva was individually transferred to a Petri dish containing the number of prey of the corresponding predator-prey

combination. At least 15 replicates were tested for each combination of predator and prey. After 24 h, the number of dead and live prey were counted.

To check for any natural mortality of the prey in the absence of the predator, 6-10 replicates were considered as controls, using the same prey density of the corresponding experiment (Table 7.1), but without predator.

Table 7.1. Number of prey offered for each combination of predator (*C. externa* larvae) and prey (eggs or nymphs of *L. hyalinus* or *N. simulans*).

Prey		Predator: <i>C. externa</i>			
Species	Life-stage	1 st instar	2 nd instar	3 rd instar	
<i>L. hyalinus</i>	Egg		30	30	30
		N1	5	15	50
		N2	5	10	30
	Nymph	N3	5	5	20
		N4	5	5	10
		N5	5	5	5
<i>N. simulans</i>	Egg		20	50	160
		N1	10	40	120
		N2	10	20	60
	Nymph	N3	5	10	35
		N4	5	5	20
		N5	5	5	10

7.2.2.2. Effect of prey on development

Larval development of *C. externa* fed on nymphs (first to early second instar) of *L. hyalinus* and *N. simulans* was studied, as compared to *S. cerealella* eggs as a factitious prey.

From 77 to 100 first instars (< 2 h old, which corresponds with a brief feeding period on *S. cerealella* eggs) of *C. externa* were individually caged in 5-cm plastic Petri dishes and offered prey ad libitum. In the treatments with *L. hyalinus* and *N. simulans* as prey, a single fresh grain of corn was offered as a source of water and food for the heteropterans. Development and survival were daily monitored to determine the larval and pupal period. Newly emerged lacewing adults (< 12 h old) were sexed and weighed using a Mettler Toledo AL204 balance (Mettler-Toledo Group, China).

7.2.2.3. Prey preference

Third instar larvae of *C. externa* were tested in prey preference experiments. Newly emerged first instar lacewing larvae were individually housed in 5-cm Petri dishes and fed with *S. cerealella* eggs until 24 h before the experiment. Newly moulted 3rd instars of *C. externa* (6-12 h old) were starved for 24 h and then each larva was individually transferred to a larger Petri dish

(9 cm diameter, 1.5 cm high, also lined with white cardboard) where they were provided with the following prey combinations: (1) *N. simulans* and *L. hyalinus*; (2) *M. euphorbiae* and *N. simulans*; (3) *M. euphorbiae* and *L. hyalinus*.

Each combination had 20 to 30 replicates and 5 controls without predators. Based on preliminary assays, the number of nymphs of each species offered in each combination was 40 and the experimental period was set to 12 h allowing a sufficient level of predation with minimal natural mortality of the prey. Per replicate, individual third instars of *C. externa* were offered 40 nymphs of similar size of each studied prey species: for *L. hyalinus*, these were 1-day-old first instars; for *N. simulans*, 3-day-old first instars; and for *M. euphorbiae*, <2-day-old nymphs (i.e. late first to early second instar). For the aphid/heteropteran prey combinations, two fresh grains of corn were placed in one side of the Petri dish, to feed *L. hyalinus* or *N. simulans*, and a piece of lettuce midrib (of about 6 cm long and 2 cm wide) with the colony of the aphids at the opposite side; for the *L. hyalinus/N simulans* combination, two grains in one side and two in the opposite were placed.

After 12 h, the numbers of killed and live nymphs were counted under a binocular stereoscope (Carl Zeiss, Stemi 508 LAB, Zeiss, Jena, Germany).

7.2.3. Statistical analysis

All statistical analyses were performed using R software, version 3.4.2 (R Core Team, 2020) and all tests were analyzed at a significance level of $\alpha = 0.05$.

Predation rates of *C. externa* larvae on immatures stages of *L. hyalinus* and *N. simulans* were analyzed using a generalized linear model, with a Poisson distribution and groups were identified by the Tukey contrasts test.

Data of developmental time that was normally distributed and homoscedastic, as indicated by Shapiro Wilk and Bartlett test, respectively, was analyzed using analysis of variance (ANOVA). Means were separated using a Tukey test. When data was not normally distributed, the non-parametric Kruskal Wallis test was used to compare multiple treatments (prey), followed by a Fisher's least significant difference test as a post hoc test, or Mann-Whitney U test to compare two treatments (prey). The percent of lacewing larvae and pupal survival was compared by means of a logistic regression and groups were identified by the Tukey test. Sex ratios were evaluated versus an equal male:female distribution (1:1 ratio) by way of a non-parametric Chi-square test.

Prey preference was analyzed by means of Manly's preference index calculated with the formula (Manly, 1974; Huang & Enkegaard, 2010):

$$\beta = \frac{\text{Log} \left(\frac{e_1}{A_1} \right)}{\text{Log} \left(\frac{e_1}{A_1} \right) + \text{Log} \left(\frac{e_2}{A_2} \right)}$$

Where β is the preference to prey species 1, e_1 and e_2 are the numbers of prey species 1 and species 2 alive after the experiment, A_1 and A_2 are the numbers of prey species 1 and prey species 2 offered to the predator. An index value close to 1 indicates a preference for prey species 1 by the predator, while an index value close to 0 indicates a preference for species 2. Significant differences between the preference indices and the value 0.5 (meaning no preference) were analyzed by a one sample *t*-test. Prey species 1 were chosen as follows: *M. euphorbiae* in the prey combination with *N. simulans* or *L. hyalinus* and *N. simulans* in the prey combination with *L. hyalinus*.

7.3. Results

7.3.1. Predation rates

Natural mortality of *L. hyalinus* and *N. simulans* nymphs observed in the control groups over a 24-h period was zero. No predation on *L. hyalinus* eggs was observed for any of the larval instars of *C. externa*. On the other hand, predation upon *N. simulans* eggs significantly increased as a function of lacewing instar ($\chi^2 = 2297.4$, $p < 0.001$) (Table 7.2). Predation upon all nymphal instars of *L. hyalinus* and *N. simulans* by lacewing larvae significantly increased from the first to the third instar ($p < 0.001$) (Table 7.2).

First instars of *C. externa* killed more first instars of *L. hyalinus* than older prey instars ($\chi^2 = 83.68$; $p < 0.001$). The number of third to fifth instar nymphs killed was similar ($p > 0.05$). Likewise, a larger number of younger instars of *N. simulans* was killed as compared to the older ones ($\chi^2 = 322.85$; $p < 0.001$). Despite that, the number of third and fourth instar nymphs killed by the lacewings, as well as the number of fourth and fifth instars, was similar (Table 7.2).

Second and third instars of *C. externa* significantly killed more younger nymphs than older nymphs for both *L. hyalinus* ($\chi^2 = 633.17$; $p < 0.001$; $\chi^2 = 1430$; $p < 0.001$) and *N. simulans* ($\chi^2 = 1112.7$; $p < 0.001$; $\chi^2 = 3799.8$; $p < 0.001$) (Table 7.2).

Table 7.2. Predation rates (means \pm SE), expressed as the number of prey killed in 24 h, by different larval instars of *C. externa* on egg and nymphal stages of *L. hyalinus* and *N. simulans*.

Prey		<i>C. externa</i>			χ^2	
Species	Life-stage	1 st instar	2 nd instar	3 rd instar		
<i>L. hyalinus</i>	Egg		0 \pm 0 (20)	0 \pm 0 (30)	0 \pm 0 (30)	No applicable
		N1	2.83 \pm 0.78Ca (23)	13.87 \pm 3.34Ba (30)	39.64 \pm 4.39Aa (25)	967.7
	Nymph	N2	1.47 \pm 0.68Cb (30)	6.65 \pm 1.53Bb (23)	21.42 \pm 3.14Ab (31)	675.4
		N3	0.67 \pm 0.55Cc (30)	3.47 \pm 0.52Bc (15)	12.07 \pm 1.16Ac (15)	286.5
		N4	0.47 \pm 0.52Cc (15)	1.31 \pm 0.47Bd (29)	5.68 \pm 1.08Ad (31)	143.5
		N5	0.07 \pm 0.26Bc (15)	0.62 \pm 0.49Be (30)	3.73 \pm 0.80Ae (33)	127.3
<i>N. simulans</i>	Egg		9.47 \pm 1.96Ca (15)	36.61 \pm 6.57Ba (18)	138.27 \pm 13.17Aa (15)	2297.4
		N1	7.44 \pm 0.86Cab (18)	25.84 \pm 4.52Bb (19)	97.67 \pm 9.90Ab (15)	1715.9
	Nymph	N2	3.40 \pm 0.91Cc (15)	14.61 \pm 2.00Bc (18)	50.27 \pm 6.94Ac (15)	816.2
		N3	1.60 \pm 0.51Cd (15)	6.83 \pm 0.92Bd (18)	28.82 \pm 3.15Ad (17)	547.3
		N4	0.73 \pm 0.46Cde (15)	3.53 \pm 0.74Be (15)	17.67 \pm 2.19Ae (15)	339.9
		N5	0.07 \pm 0.26Ce (15)	1.47 \pm 0.52Bf (15)	6.79 \pm 1.93Af (19)	161.5

Different lowercase letters within a row, or uppercase letters within a column and a prey species indicate significant differences at $\alpha = 0.05$. The number of replicates (per predator/prey combination) is given in parentheses.

7.3.2. Effect of prey on development

Prey species significantly affected immature survival of *C. externa* ($\chi^2 = 140.02$; $p < 0.001$). Survival up to the adult stage of the lacewing was highest when feeding on the factitious prey (*S. cerealella* eggs) and lowest when feeding on *N. simulans* nymphs (Table 7.3).

Survival of *C. externa* when presented with *L. hyalinus* was more affected in the pupal stage ($\chi^2 = 43.97$; $p < 0.001$), and only 53.6% of the initial number of individuals tested survived to the adult stage. However, survival of the lacewing when offered *N. simulans* was significantly and gradually affected from the first instar to the pupal stage ($\chi^2 = 102.93$; $p < 0.001$) and only 10.3% of the initial number of individuals survived to the adult stage. Survival of the lacewing with *S. cerealella* eggs was high and similar in all larval instars and in the pupal stage ($\chi^2 = 0.49$; $p = 0.919$), and 93% of the individuals reached the adult stage (Figure 7.1, Table 7.3).

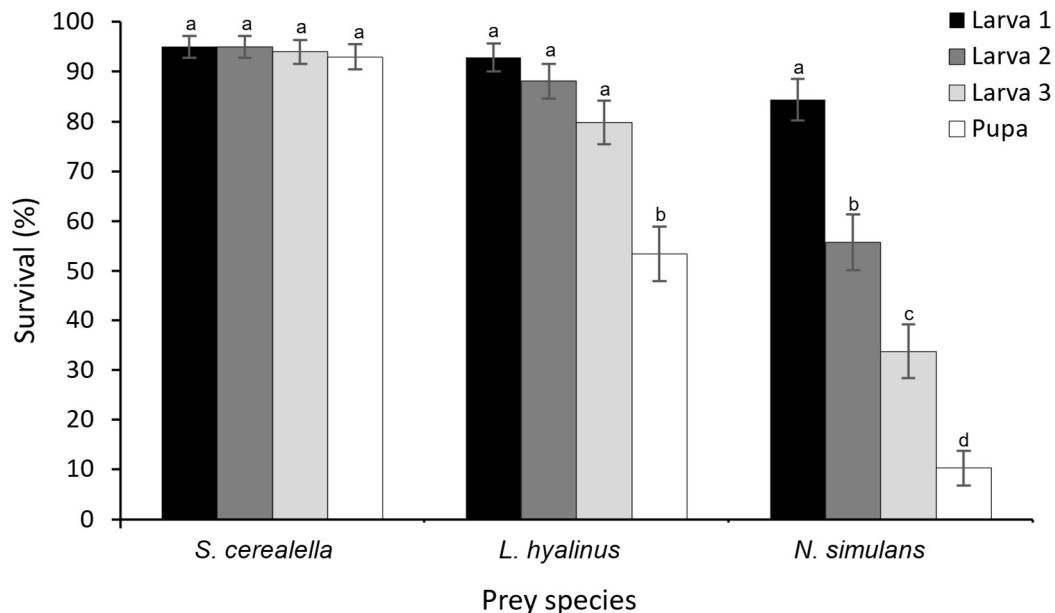


Figure 7.1. Survival during larval and pupal development of *Chrysoperla externa* fed with *Sitotroga cerealella* eggs, or nymphs of *Liorhyssus hyalinus* or *Nysius simulans*. Different letters within a treatment indicates significant differences at $\alpha = 0.05$.

No differences in the larval period were found among *C. externa* males fed on *L. hyalinus* nymphs and *S. cerealella* eggs, but offering *N. simulans* as prey yielded a significantly shorter male larval period ($\chi^2 = 17.55$, $df = 2$, $p < 0.001$). Larval period of lacewing females was similar when fed with *L. hyalinus* nymphs and *S. cerealella* eggs ($W = 540$, $df = 1$, $p = 0.751$). Since only two lacewing females reached adulthood when offered *N. simulans*, this data was excluded from the analysis (Table 7.3).

Male pupal period of the lacewing was longer with *L. hyalinus* as prey and shortest with *N. simulans* ($\chi^2 = 15.89$, $df = 2$, $p < 0.001$). Female pupal period was longer on with *S. cerealella* eggs than on *L. hyalinus* nymphs ($W = 882.5$, $df = 1$, $p < 0.001$) (Table 7.3).

Male and female adult weights were heaviest with *S. cerealella* as prey, and there were no differences in lacewing male weights when fed on *L. hyalinus* and *N. simulans* (males: $F = 19.54$, $df = 2$, $p < 0.001$; females: $F = 47.07$, $df = 1$, $p < 0.001$). Sex ratios of *C. externa* did not significantly deviate from a 1:1 ratio, with either *S. cerealella* ($\chi^2 = 2.42$, $p = 0.119$) or *L. hyalinus* ($\chi^2 = 3.76$, $p = 0.053$) as prey (Table 7.3).

Table 7.3. Developmental parameters (means \pm SE) of *C. externa* on different prey species.

Prey species	Survival (%)	Larval period (days)		Pupal period (days)		Adult weight (mg)		Sex Ratio (♂:♀)
		Males	Females	Males	Females	Males	Females	
<i>S. cerealella</i>	93.0 \pm 2.6a (100)	11.19 \pm 0.38a	11.18 \pm 0.36a	10.78 \pm 0.35b	10.94 \pm 0.33a	6.86 \pm 0.69a	7.40 \pm 0.14a	1:0.72
<i>L. hyalinus</i>	53.6 \pm 5.4b (84)	11.42 \pm 0.54a	11.45 \pm 0.66a	10.95 \pm 0.43a	10.57 \pm 0.37b	5.82 \pm 0.70b	7.14 \pm 0.81b	1:1.81
<i>N. simulans</i>	10.3 \pm 3.5c (77)	9.71 \pm 0.42b	11.25 \pm 0.35*	10.08 \pm 0.49c	10.25 \pm 1.06*	5.68 \pm 0.57b	8.42 \pm 0.72*	1:0.33*

Different letters within a column indicate significant differences at $\alpha = 0.05$: Tukey contrast test (survival), Kruskal Wallis test (larval and pupal male period), Mann-Whitney U test (larval and pupal female period); Tukey's HSD test (adult weight).

The initial number of first instars tested is given in parentheses.

* Data excluded from the analysis given the low number of females surviving.

7.3.3. Prey preference

Third instar larvae of *C. externa* preferred *N. simulans* to *L. hyalinus* ($\beta = 0.76 \pm 0.09$), and *M. euphorbiae* to *L. hyalinus* ($\beta = 0.86 \pm 0.06$) or *N. simulans* ($\beta = 0.71 \pm 0.09$). All preference index values were significantly different from 0.5 (Table 7.4). Natural mortality in 12 h was 3.3 % for *M. euphorbiae*, whereas there was no control mortality for *L. hyalinus* and *N. simulans*. As natural mortalities of the prey in 12 h were lower than 5%, observed values were not corrected (Bonte et al., 2015).

Table 7.4. Number (means \pm SE) of *L. hyalinus*, *N. simulans* and *M. euphorbiae* nymphs killed in 12 h by third instars of *C. externa* and prey preference index (β , mean \pm SE) calculated for different prey combinations

No. of prey killed		No. of replicates	β	t	p-value
<i>N. simulans</i>	<i>L. hyalinus</i>	30	0.76 \pm 0.09	16.35	< 0.001
24.83 \pm 6.94	9.63 \pm 4.02				
<i>M. euphorbiae</i>	<i>L. hyalinus</i>	21	0.86 \pm 0.06	27.72	< 0.001
29.00 \pm 4.88	7.86 \pm 4.04				
<i>M. euphorbiae</i>	<i>N. simulans</i>	20	0.71 \pm 0.09	9.28	< 0.001
28.10 \pm 4.45	16.50 \pm 6.95				

Preference index value (β) tested for difference from 0.5 with a one-sample t-test at a significant level $\alpha = 0.05$.

7.4. Discussion

To date, no studies have addressed the potential of *C. externa* to suppress pests in quinoa. The present laboratory study comprises the first effort to explore the role of *C. externa* for conservation or augmentative biological control of *L. hyalinus* and *N. simulans*, serving to manage population densities of these two emerging pests of quinoa.

Chrysoperla externa was not able to feed on the eggs of *L. hyalinus* in any of the larval ages, probably due to the inability to penetrate the chorion with its mandibles; when examined under the stereoscope, larvae of the lacewing did try to pierce the *L. hyalinus* eggs but without success of feeding or inflicting damage, with pest nymphs successfully hatching. This was not the case for *N. simulans* eggs, which were effectively consumed by all larval instars of the predator.

All instars of the lacewing were able to kill individuals of the different nymphal ages of *L. hyalinus* and *N. simulans*. Larvae of the predator became more voracious with increasing instar as seen in other studies (Bastidas et al., 2010; Tavares et al., 2011; Fonseca et al., 2015; Cuello et al., 2019; Luna-Espino et al., 2020). For instance, the third instar larvae killed more than three times

the number of prey killed by the second instar. This is in line with Canard (2007) who stated that third instar larvae of lacewings account for the major part of the total larval prey consumption; for instance, third instars of *C. carnea* killed between 72 to 80% of the total number of prey killed during the larval stage, when they were offered *Tetranychus urticae* Koch (eggs), *Mamestra brassicae* (L.) (eggs or first instars), *Myzus persicae* (Sulzer) (second instars), *Ephestia kuehniella* Zeller (eggs) or *Pectinophora gossypiella* (Saunders) (eggs or first instars) as prey.

Second and third instar larvae killed a significantly larger number of young instars than older instars, both of *L. hyalinus* and *N. simulans*. However, first instars of *C. externa*, were able to successfully catch only first and second instars of *L. hyalinus*, or first to third instar of *N. simulans*, whereas older nymphs of both pests easily escaped from the smaller lacewing larvae. These older nymphs were often attacked by being pierced at the tarsus. Similarly, smaller individuals of different prey types were reportedly killed in higher numbers by *C. externa* than their larger conspecifics (Pacheco-Rueda et al., 2015). Further, *C. externa* killed a larger number of *N. simulans* nymphs than *L. hyalinus* nymphs, which may be primarily due to the overall larger size of *L. hyalinus* as compared with *N. simulans*, although other factors may have also affected the prey consumption observed (e.g. mobility).

At 26 °C, *L. hyalinus* and *N. simulans* have an oviposition period of about two months with an average oviposition rate of 4 to 5 eggs per day (unpublished data). The average predation rates of third instar *C. externa* observed in the present study (39.6 and 97.6 first instars nymphs of *L. hyalinus* and *N. simulans*, respectively), exceed by far the fertility rates of individual females of these pests under laboratory conditions. This indicates a promising perspective for use of *C. externa* in augmentative biological control programs during peak numbers of the heteropterans in quinoa. However, even for a highly acceptable prey in the laboratory there may be a different outcome as to the predation rate under field conditions, where a complex of ecological interactions is expected to affect the performance of an insect predator (Canard, 2007).

Although lacewing larvae avidly fed on first and second instars of both *L. hyalinus* and *N. simulans*, these species were not as suitable as prey to support the larval development as were the eggs of *S. cerealella*. This may in part be attributed to the nutritional value of the lepidopteran eggs (Albuquerque et al., 1994; López-Arroyo et al., 1999; Pappas et al., 2007; Huang & Enkegaard, 2010) and more in particular the efficiency of *S. cerealella* eggs as a factitious food for *C. externa* has been widely documented (McEwen & New, 2007; Haramboure et al., 2015; Bezerra et al., 2017). The worst prey for the larval development of the lacewing appeared to be *N. simulans* nymphs, with only 10.3% of immature survival of the lacewing and

high mortality in both the larval and pupal stage. Unsuitability of prey for growth and survival was also observed for *Chrysoperla rufilabris* (Burmeister), whose larvae voraciously consumed individuals of *Tetranychus gloveri* Banks offered in the laboratory, but they could not support the full development of the lacewing (Canard, 2007). Likewise, *Chrysoperla carnea* (Stephens) and *C. rufilabris* reared on *Drosophila melanogaster* Meigen larvae suffered high mortality in the larval and pupal stages (Hydorn & Whitcomb, 1979; Osman & Selman, 1996).

The unsuitability of the studied heteropteran prey for growth and survival of *C. externa* observed in our laboratory study does not necessarily reduce its potential to suppress populations of these true bugs, because the predator may complement its nutritional requirements for optimal development on other prey (McEwen & New, 2007). However, in an augmentation program, inundative releases of *C. externa* larvae might be more suitable than inoculative releases (Senior & McEwen, 1998)

Third instars of *C. externa* showed a clear preference for the aphid *M. euphorbiae* over both *L. hyalinus* and *N. simulans*, when they were offered in two-prey combinations. Preference for aphid prey was also reported in other species of green lacewings (Ables et al., 1978; Ding & Chen, 1986; Nordlund & Morrison, 1990; Huang & Enkegaard, 2010). Although aphids have been demonstrated to be suitable for larval development of lacewings, the reason for these reported preferences is not clear and may be attributed to physical attractiveness determined by such factors as size, colour, mobility or chemical cues of the prey, more than a perception of their nutritional value (El-Arnaouty et al., 1996; Cardoso & Lazzari, 2003; Canard, 2007; Huang & Enkegaard, 2010; Garzón et al., 2015; Gamboa et al., 2016). This is supported by the fact that green lacewing larvae preferred *N. simulans* over *L. hyalinus* nymphs, although the former were found to be the worst prey for growth and survival of *C. externa*. In addition, Canard (2007) stated that the discovery of the prey by lacewings is random but can be slightly stimulated, within a very short distance, by the honeydew of sap-sucking insects such as aphids, mealybugs or other species of Sternorrhyncha.

Macrosiphum euphorbiae is an aphid species that infests quinoa fields in the lowlands of Peru throughout the crop phenology, with peak numbers during the vegetative stage and decreasing populations towards the end of the cropping season. The aphid population is regulated by species from the aphidophagous guild that usually appear in large numbers, such as Aphidiinae wasps, lady beetles, and hoverfly larvae besides green lacewings (Chapter 4). On the other hand, *L. hyalinus* and *N. simulans* start the infestation during the grain filling stage, but peak numbers are found at the maturation stage, not coinciding with the highest infestation of *M. euphorbiae*

(Gómez & Aguilar, 2016; Latorre, 2017; Chapters 4 and 5). Moreover, being a generalist predator, the preference of *C. externa* for aphids over the heteropterans does not necessarily indicate a limited potential to suppress significant densities of *L. hyalinus* or *N. simulans* in quinoa fields. For instance, in a field-cage study on cotton, *C. carnea* larvae were able to kill substantial numbers of the lepidopterans *Chloridea virescens* (Fabricius) and *Helicoverpa zea* (Boddie) in the presence of high numbers of other prey, including the preferred prey *Aphis gossypii* Glover, in spite of the negative influence of the latter on the efficiency of the lacewing in controlling the target pests (Ridgway & Jones, 1968; Ables et al., 1978). Likewise, in another field-cage study on cotton, the presence of *A. gossypii* as alternative prey did not significantly affect the predation by the lacewing *Mallada signatus* (Schneider) on larvae of *Helicoverpa armigera* (Hübner) (Bahar et al., 2013).

Given the results of the present predation experiments and the fact that *C. externa* naturally occurs in quinoa fields (Valoy et al., 2015; Cruces et al., 2016), this predator might be a target for conservation biological control programs. Moreover, as *C. externa* is easily mass reared and commercially available in Peru (e.g., by SENASA), the lacewing might also be considered for augmentation biological control programs, particularly during the late crop phenology when pest density is expected to be high. Field studies are needed, however, to better understand the potential of *C. externa* as a biological control agent in quinoa and to determine the most suitable strategies, taking into account the complexity of the quinoa agroecosystem.

Chapter 8

General discussion and future perspectives

With increasing worldwide concerns about food security, there is an ever rising demand for healthy and nutritional food (Alandia et al., 2020; Angeli et al., 2020). Over the last decade, the nutritional properties of Andean quinoa and its specific agronomic qualities (e.g. drought resistance) have been revalued. As a result, the demand for quinoa grains started to steadily increase, leading to a significant expansion of the cultivated area in the South American countries of Ecuador, Chile and particularly Bolivia and Peru (the main quinoa producers) (Cruces et al., 2016; Bedoya-Perales et al., 2018a; Hinojosa et al., 2021; Chorbadjian et al., 2021). Thus, new quinoa production areas emerged both inside and outside the Andean region (Gómez-Pando et al., 2014; Gómez & Aguilar, 2016; Alandia et al., 2020).

In Peru, two of the new quinoa production zones correspond to the Coast (< 500 m a.s.l.) and the “Maritime Yunga” (500 – 2,300 m a.s.l.) ecoregions (Gómez-Pando et al., 2014; Chapter 4). In these areas, more advanced cultivation practices are applied (i.e. technified irrigation, use of machinery, chemical fertilizers and pesticides) allowing to obtain higher yields of quinoa than in the highlands (Gómez-Pando et al., 2014). However, a greater incidence of pests may limit the quinoa production in these new zones to a higher degree than in the highlands (Cruces et al., 2016; Latorre, 2017).

The present research mainly focused on the entomofauna associated with quinoa cultivated in the new production zones of the Peruvian lowlands. We investigated the insect community and major insect pests of quinoa, as well as alternatives to chemical pest control in order to set a basis for more sustainable strategies of pest management, based on the principles of an Integrated Pest Management (IPM) (Malavolta et al., 2005; Joas & Cotillon, 2009; Barzman et al., 2015). Our study is the first effort to try to understand the insect community of quinoa from the coastal areas of Peru, where there is a promising perspective to produce quinoa at a large scale.

In our explorative field studies (chapters 3 and 4), carried out from September 2015 to September 2016, we examined the entomofauna associated to quinoa cultivation in two field sites belonging to new zones of quinoa production, La Molina (at 244 m a.s.l.) and Majes (at 1,410 m a.s.l.), as well as a site in San Lorenzo (at 3,311 m a.s.l.) that belongs to a traditional quinoa production zone in the Andes. Our surveys revealed that the quinoa moth, *Eurysacca melanocampta*, the key pest of quinoa in the highlands, is also present at lower elevations (Majes and La Molina) as were the serpentine leafminer, *Liriomyza huidobrensis*, and the western flower thrips, *Frankliniella occidentalis*. However, the heteropteran pests *Liorhysus hyalinus* and *Nysius simulans* were only found at the La Molina and Majes field sites. Our

laboratory studies on the thermal biology of these heteropterans (chapter 6), indicated that they did not develop well at 18 °C and 22 °C. Since most of the highlands of Peru are characterized by overall lower temperatures, these findings explain the absence of *L. hyalinus* and *N. simulans* in the coolest parts of the Andean region. For example, in Jauja (over 3,000 m a.s.l.) the annual minimum temperature is about 4.5 ± 3.4 °C whereas the maximum averages 19.5 ± 2.2 °C (SENAMHI, 2021).

Based on pitfall trapping data, we found that the field site from the Andes, in San Lorenzo, had the highest overall insect diversity (Shannon index = 3.6; Margalef index = 20.2) and the highest species richness of natural enemies (Margalef index = 10.3) (Chapter 3). Moreover, the calculated estimators (ACE, Chao1, Chao2) indicated that it is possible to find even a higher species richness (between 29 to 38% more) than that observed in our survey. At the coastal level, at the La Molina field site, intermediate values of insect diversity (Shannon index = 2.4; Margalef index = 11.1) and species richness of natural enemies (Margalef index = 5.5) were found. One limitation of this study was that only one field per location was monitored and therefore such diversity indices might not be fully representative of the corresponding locality. However, considering the complex vegetation in most of the Peruvian agricultural valleys (e.g. as a result of different irrigation and management regimes, different nearby crops) and the instability of annual crops such as quinoa, which is mostly cultivated at small-scale (i.e. crop plots <2 ha), it may not be feasible to find a representative value of insect diversity for quinoa at each locality, even if more field sites would be evaluated. However, given the agroecological traits of both localities and the Peruvian ecoregions, a higher diversity is expected in the highlands than in the coastal areas of Peru (Gómez-Pando et al., 2014; Rojas & Patiño, 2014; Gómez & Aguilar, 2016).

On-plant samplings (Chapter 4) showed that pest incidence in quinoa was higher in La Molina than in San Lorenzo, where only the key quinoa pest *E. melanocampta* was collected at high densities (up to 15 larvae per plant) (Villanueva, 1978; Blanco, 1994); other pests were found in small numbers, which is in line with the literature (Bazile et al., 2014; Saravia et al., 2014). This suggests that the IPM programs for quinoa in the highlands should be more focused on establishing strategies to manage *Eurysacca* quinoa moths, which will also contribute to keep other lepidopteran pests under control (e.g. *Helicoverpa quinoa*, *Copitarsia* spp.) (Quispe et al., 2014; Saravia et al., 2014).

At the coastal level (La Molina), *E. melanocampta* was found at even higher densities than in the highlands (with up to 65 larvae per plant). Besides, *Macrosiphum euphorbiae* and *L. huidobrensis*

were collected at relatively high densities. These pest species, alongside the thrips *F. occidentalis*, as well as the true bugs *L. hyalinus* and *N. simulans*, will likely become an important barrier for successful quinoa production in the lowlands, as documented by Gómez & Aguilar (2016), Cruces et al. (2016) and Latorre (2017). However, significant numbers of natural enemies were sampled in the field, including hymenopteran parasitoids of leafminers and aphids, hoverfly larvae, predatory bugs, carabids and lacewing larvae, which may play an important role in IPM strategies (Chapter 4).

Based on our study and on other published information, there is a potential for implementing biological control strategies as part of an IPM program in quinoa, both in the Andean zone and in the lowlands, given that arthropod natural enemies (predators and parasitoids) constitute a considerable part of the quinoa entomofauna. For example, in the Andean region, parasitism of *E. melanocampta* in quinoa may reach up to 25% during the beginning of the grain filling, up to 45% during the milk grain stage, and up to 80% during the maturation of the grains (Mamani, 1998). The main larval parasitoids of *Eurysacca* moths are reported to be an unidentified species of the genus *Phytomyptera* (Tachinidae) and another of the genus *Diadegma* (Ichneumonidae), as well as *Copidosoma gelechia* Howard (Encyrtidae) (Rasmussen et al., 2003; Saravia et al., 2014). For the lowlands of Peru, there are no current data on parasitism of *E. melanocampta*, but we did observe parasitism on other pests: for *L. huidobrensis* leafminers there was up to 100% of parasitism, mainly by *Halticoptera* spp. (Pteromalidae) and *Chrysocharis* spp. (Eulophidae), whereas for *M. euphorbiae*, only 2.5% of parasitism was observed, and the main collected parasitoids were *Lysiphlebus testaceipes* (Cresson), *Aphidius matricariae* (Dalman) and *Aphidius colemani* (Dalman) (Braconidae) (Chapter 4).

Among the insect predators, species of the family Coccinellidae and Chrysopidae are noted to have a more significant role in pest control (Valoy et al., 2015). *Chrysoperla externa* has been reported to reach its highest population densities coinciding with the occurrence of *Eurysacca* species (González Olazo & Reguilón, 2002; Valoy et al., 2015). Among the coccinellid species, *Hippodamia convergens* and *Eriopis connexa* have been reported during peak numbers of aphids (Valoy et al., 2011). Likewise, in our study, relatively large numbers of the syrphid *Allograpta exotica* larvae have been noted during high infestations of *M. euphorbiae* in La Molina (Chapter 4).

Given the diversity of beneficial insects observed in La Molina and San Lorenzo, Conservation Biological Control (CBC) programs can be feasible in these localities. This type of biological control involves, in general terms, a series of practices that enhance the preservation of the

established natural enemies in the crop and promote their population increase by means of habitat diversification and by minimizing agricultural practices that are detrimental to the natural enemies. Thus, providing a better environment for the natural enemy complex in the agroecosystem is expected to contribute to the pest population regulation by arthropod predators and parasitoids (Begg et al., 2017; Cloyd, 2020).

One of the practices that fosters biodiversity preservation is mixed cropping, which is applied in the traditional quinoa production systems in the Andes. Promoting the diversity of crops and non-crop plants in a valley also promotes biodiversity and its associated ecosystem services. For instance, in Cusco, Peru, the number of parasitoid species of *E. melanocampta* in the quinoa fields increased with the diversity of the surrounding areas (Costa et al., 2009a, 2009b). Furthermore, since organic quinoa is produced in the highlands of Peru, establishing CBC strategies may improve organic production in that zone (Wolfe, 2002; Begg et al., 2017; Cancino-Espinoza et al., 2018; Muneret et al., 2018; Cloyd, 2020).

Unlike the production sites in the Andes, the agricultural valleys at lower elevations consist of a few crops, mostly cultivated as monocultures. Such crops are mainly managed under a conventional cropping system, with the recurrent use of chemical pesticides. Although a relatively high number of natural enemies may visit the quinoa fields, partly due to the abundant pollen produced by the quinoa plants, these potential biological control agents are affected by the agrochemicals applied in quinoa and in the surrounding crops. As such, during pesticide treatments, natural enemies cannot find any temporal refuge, minimizing their potential establishment in the fields. For this reason, the protection of the functional biodiversity should focus on a reduction of agrochemical impacts on insect diversity by promoting the use of environmentally friendlier pesticides and by establishing economic thresholds levels (ETLs) in quinoa (Saravia et al., 2014; Latorre, 2017; Chapters 3 and 5). Minimizing the impact of agrochemicals on insect diversity, and particularly the diversity of natural enemies, is a key CBC strategy, but it might have a greater impact if applied at a large scale (e.g. in an entire valley) rather than in a single quinoa field (Wilby & Thomas, 2002; Maredia et al., 2003; Mahmood et al., 2016). Another important aspect of CBC to consider is the conservation of natural and semi-natural habitats, which may serve as a refuge for the natural enemies (Holland et al., 2016).

On the other hand, the at first glance low insect diversity values (Shannon index = 1.6; Margalef index = 7.9) and low species richness of natural enemies (Margalef index = 3.85) found in Majes might seem unpromising for the implementation of CBC strategies at this locality (Chapter 3). However, the studied field site was subjected to an intense use of broad-spectrum insecticides

belonging to the pyrethroid group which are known to be detrimental to both phytophagous insects and their natural enemies. Therefore, more field studies are needed to determine the potential occurrence and diversity of the natural enemies in quinoa fields in this zone, and with this to fully understand their function in such agroecosystems (Stanley & Preetha, 2016).

The monitoring of phytophagous insects of quinoa at the field site in Majes revealed a similar pest complex to the coastal field site in La Molina, although their natural incidence and density could not be accurately evaluated due to the intensive use of pyrethroids. This broad-spectrum insecticide type likely was the main factor that kept all insect populations, including those of the natural enemies, at low numbers during the first stage of the crop phenology. In this context, the pyrethroids used likely eliminated the key biological control agents of *F. occidentalis*, the only pest species that infested the crop in significantly large numbers (i.e. 191 trips per plant) as the insecticide treatments ceased. Likewise, natural enemies started to colonize the crop once the insecticide treatments stopped, and more in particular the aphidophagous guild (aphidiinae wasps, coccinellids and chrysopid larvae) was observed to reappear (Chapter 4). Moreover, since pyrethroid insecticides are of general use in Majes and they are applied without a rotation system of active ingredients with a different mode of action, *F. occidentalis* (and other pests) likely may have developed resistance to insecticides belonging to this group (Croft & Whalon, 1982; Cisneros, 2012; Hénault-Ethier, 2015; Sparks & Nauen, 2015).

To better understand the impact of insecticide use on the natural enemy complex in quinoa, we conducted a field study in La Molina in which the effect of four insecticides on the abundance of both target and non-target arthropod fauna in quinoa was evaluated (Chapter 5). In this study, the pyrethroid cypermethrin had a short- as well as long-term negative effect on predatory true bugs (*Nabis capsiformis*, *Metacantus tenellus*, *Rhinacloa* sp.), syrphid larvae (*Allograpta* sp.) and chrysopid larvae. Such detrimental side effects of cypermethrin on non-target species are widely documented in the literature (Ishaaya & Degheele, 1998; Desneux et al., 2007; Stanley & Preetha, 2016; Jeschke et al., 2019).

In the abovementioned field study carried out in La Molina in 2017 (Chapter 5), *E. melanocampta* did not infest the plots as this moth did during our survey in 2015. One explanation for this may be, in part, that during 2015 in La Molina, there were two growing seasons of quinoa: the first from January to May, and the second from September 2015 to January 2016, both with a high incidence of *E. melanocampta* larvae (Luz Gómez, personal communication). During 2017, however, there was only one cropping season of quinoa (from July to December), with a fallow period of 4 months, and during this campaign the lepidopteran that infested the plots was

Spoladea recurvalis, but not on a recurring basis. Further research is needed to fully understand the factors that limit or favor infestation by *E. melanocampta* as well as *S. recurvalis* in quinoa fields under coastal conditions.

Based on the field evaluation of imidacloprid, cypermethrin, teflubenzuron and emamectin benzoate on target pests and their side effects on non-target species (Chapter 5), the two latter insecticides are deemed to be the most promising active ingredients to be used against *S. recurvalis* larvae, due to the lesser negative effects on the natural enemy complex as compared with imidacloprid or cypermethrin. With a more favourable environmental profile, these selective insecticides may be included in IPM programs for quinoa cultivated in the coastal areas (Hardy, 2011; Amor et al., 2012; Govindan et al., 2013; Torres & Bueno, 2018). However, since only one cropping season was monitored, complementary field studies in different areas and cropping seasons are needed to accurately determine the efficacy of teflubenzuron and emamectin benzoate in controlling *S. recurvalis* as well as their effects on the beneficial fauna, in order to recommend a more general use of these insecticides in quinoa (Hassan, 1985; FAO, 2006).

On the other hand, foliar application of imidacloprid was found to be harmful to the entomofauna of quinoa, affecting not only the abundance of the key natural enemy species, but also negative effects on the overall arthropod diversity and the species composition. Such side effects of imidacloprid have been extensively reported involving a wide range of natural enemies, including the aphidophagous guild (Delbeke et al., 1997; Khani et al., 2012; Zhang et al., 2016; Kar, 2017; Amjad et al., 2018; Mohammed et al., 2018; Martínez et al., 2019). Moreover, based on the pitfall trap data, diversity of the ground dwelling species was also significantly affected. Although this compound had a good performance in the control of *S. recurvalis*, *M. euphorbiae*, *F. occidentalis* and *N. simulans*, we conclude that imidacloprid is not suitable for IPM programs, as the use of this active ingredient is expected to limit the impact of the natural enemy complex on the main pests of quinoa (Funderburk et al., 2013).

The heteropteran pests *L. hyalinus* and *N. simulans* occurs in the lowlands of Peru and have been noted to cause serious problems at the grain filling stage. Our laboratory study on the thermal biology of these species (Chapter 6) indicated that the heteropterans are better adapted to warmer temperatures, with 30 °C as an optimal temperature at which the highest fecundity and high egg viability were observed. Poor reproductive parameter values were observed at 22 °C and at 18 °C nymphs of the heteropterans did not successfully develop. As noted above, environmental thermal conditions might limit the occurrence of the true bugs in the Peruvian

highlands (Chapter 3 and 4). However, in certain localities in the Andean region located at lower altitudinal limits (i.e. Cajamarca, at 2,500 – 2,700 m a.s.l.) there are warmer temperatures and less adverse climatic conditions as compared with the higher parts (i.e. Puno, at 3,810 – 4,050 m a.s.l.). Therefore, more surveys along several altitudinal gradients are needed to determine the distributional limits of these heteropterans (Bazile et al., 2014; SENAMHI, 2021).

Potential occurrence of *L. hyalinus* and *N. simulans* is expected in localities along the Coast of Peru. Taking into account the thermal conditions that characterised the coastal region (SENAMHI, 2021), higher incidence of these heteropterans is expected in summer than during the rest of the year. For this reason, early sowings of quinoa (early to mid-winter) are recommended, in order to avoid that the maturation of the grains coincides with summer. Moreover, although there are varieties well adapted to warm conditions, cultivation of quinoa during the summers is less recommended because high incidence of pests is expected at that time, not only *L. hyalinus* or *N. simulans*, but also lepidopteran larvae that infest the grains, such as *E. melanocampta*, *Chloridea virescens*, *Spodoptera eridania* and *S. recurvalis* (Sánchez & Vergara, 2002; Carrera, 2013; Soca, 2021).

Based on the estimated linear model parameters, around 5 generations of *L. hyalinus* and 3 generations of *N. simulans* per year and under coastal conditions (in Lima) may be expected. However, in the field there are other factors that may also influence the development and reproduction of insects (e.g. availability and type of food, stress due to biotic and abiotic factors). As the heteropterans were studied with only one type of food (corn grains) and at constant temperatures, which do not occur in the field, deviations from our observed values may be expected. However, laboratory results like ours can be considered useful references for prediction purposes (Bonte, 2016).

An optimal use of pesticides as well as the availability of ETLs would also facilitate the implementation of Augmentative Biological Control (ABC) programs, either by using an inoculative or an inundative strategy. ABC refers to releasing mass-reared natural enemies at specific densities in order to reduce populations of target pests. These biocontrol agents may be mass reared by the farmers or in biofactories that commercially distribute them (van Lenteren, 2012; Perez-Alvarez et al., 2019).

Chrysopid species have been extensively and successfully used in ABC programs in a variety of crops (Senior & McEwen, 2007; Souza et al., 2019; Venzon et al., 2021). Taking into account that lacewings were naturally present at the later stages of the crop phenology, chrysopid species may be good candidates for the biological control of grain-feeding insects (i.e. *E. melanocampta*,

L. hyalinus or *N. simulans*) (Canard, 2007; Valoy et al., 2015). *C. externa* is a promising predator for use in ABC in quinoa in the coastal region of Peru, due to its commercial availability at SENASA (National Service of Agrarian Sanitation). Being a generalist predator, this lacewing may assist in regulating populations of aphids, thrips, small lepidopteran larvae and heteropteran nymphs that infest the quinoa fields (Canard, 2007; Garzón et al., 2015; Gamboa et al., 2016; Chapter 7). In this context, we chose *C. externa* to study its potential as biological control agent of *L. hyalinus* and *N. simulans* (Chapter 7).

Considering the substantial predation rates on the early life stages of *C. externa* on *L. hyalinus* as well as *N. simulans*, the predator may hold promise as a biological control agent in quinoa cultivated in the lowlands; lacewing larvae avidly killed significant numbers of first and second instar nymphs of these heteropterans. Therefore, we suggest that lacewings might be a target for conservation biological control programs, but also for inundative releases in the later stages of the crop phenology, during the grain filling when pest densities are expected to be high and chemical insecticides are less feasible to use due to pesticide residues issues in the harvested grains. However, considering the observed preference of lacewing larvae for aphids over *L. hyalinus* and *N. simulans*, and given the fact these species were not sufficiently suitable to support larval development of the predator as compared with *Sitotroga cerealella*, complementary laboratory and field studies are needed to determine whether *C. externa* can suppress significant numbers of the heteropterans in the presence of alternative prey under field conditions.

Based on the results of the current study, and on the information provided in the literature (Chapter 2), there is a promising perspective on implementing IPM programs in quinoa in the lowlands of Peru. However, there are aspects that need to be improved, particularly regarding pest sampling and setting up ETLs, which are essential components of an IPM. Although there are some recommendations about the monitoring of pests, they are based on information coming from other crops rather than specific studies on pest presence according to the phenology of quinoa. As to the ETL, most of the thresholds used in cropping systems in Peru, particularly in large scale farming systems, are empirical and based on the personal experience of agronomists and agricultural technicians who grow a particular crop throughout several years and cropping seasons (Cisneros, 2012). As quinoa is a new crop in the Peruvian coastal areas, there is almost no information that can be used as a reference for the establishment of ETLs, which may complicate the implementation of IPM programs in that area. In this context, studies on the seasonal phenology of the major pests in the coastal areas can generate useful basic information for setting up or finetuning ETLs.

Part of our results can be extrapolated to other South American countries where a similar pest complex has been reported (Dughetti, 2015a, 2015b; Chorbadian et al., 2021). For example, based on the lower developmental thresholds and thermal requirements of *L. hyalinus* and *N. simulans*, calculated in the present study, their potential distribution in quinoa cropping systems in South America can be estimated, particularly in countries where these heteropterans have already been reported as pests in this crop, like in Argentina and Chile. In the latter countries, however, the pest distribution at regional level is still unknown. Moreover, as *C. externa* naturally occurs in quinoa and other crops in both of the above countries (Albuquerque et al., 1994; Dughetti, 2015a), further studies on this lacewing as a biological control agent of *L. hyalinus* and *N. simulans* can be carried out. Species such as *M. euphorbiae*, *L. hyalinus*, *F. occidentalis*, *L. huidobrensis* and *S. recurvalis* that have a cosmopolitan distribution, eventually may become pests in quinoa in other parts of the world where this crop is currently being cultivated (currently in more than 120 countries). In this context, our studies can offer a useful reference for further research on the management of pests in quinoa around the world.

Eurysacca species are currently distributed in the Andean countries. However, the fact that *E. melanocampta* was found in the field site at San Lorenzo (in the highlands) and La Molina (in the lowlands), two localities extremely different in terms of environmental conditions, suggests a wide adaptability of this pest. This finding indicates that this species may represent a future threat to the export of quinoa from the Andean countries. Quinoa grains are subjected to a post-harvest process in which they are cleaned, purified and selected, minimizing the presence of pests in consignments of grains. However, this is an issue that quinoa growers and agricultural companies exporting quinoa from the Andean zone should take into consideration in order to mitigate the possible risks of shipping consignments contaminated by this pest (e.g. the moth eggs).

Lastly, cultivation of quinoa in the new production areas in Peru has been decreasing, whereas in the traditional zone, in the highlands, the production area has stayed stable. Since the cultivation of quinoa in the Andes of Peru is mainly organic and conventional production of this grain is more feasible in the lowlands, the emergence and expansion of new areas for growing quinoa in the coastal region is not expected to impair farmers from the highlands. In this context, there is a key role for the Peruvian government to establish appropriate agrarian policies to promote and improve the production of organic quinoa in the Andes and of conventional quinoa in the lowlands. Such policies may include agricultural extension programs aimed at transferring data from studies on pest management to the farmers in order for them to better integrate their farming practices, as well as technical assistance for farmers, particularly small-holders, and

advice on the appropriate use of agrochemicals. Farmer Field Schools may play an important role for successful implementation of IPM in quinoa, considering that most of the smallholder farmers may resist changing their habits and, for example, not readily shift from broad-spectrum compounds to selective insecticides which are more expensive. SENASA has a key role to play in strengthening the monitoring program for chemical residues in quinoa grains in order to prevent rejections of this grain and facilitate the expansion toward new markets. Finally, another relevant policy would be the implementation of technical regulations for organic and conventional quinoa growing, in order to establish key cultivation parameters (e.g. sowing periods per locality, pesticides allowed, appropriate varieties per zone) that would facilitate the role of agricultural extensionists in setting up IPM in this crop.

In summary, further research should be focused on the biology and ecology of other major pests of quinoa, particularly those occurring in the lowlands where information on the pest complex is still scarce. Impact of pests in terms of yield loss is still a knowledge gap for most of the species that needs to be addressed. Laboratory and field studies on predation or parasitism by key natural enemies will allow us to better understand the effectivity of the biocontrol agents in suppressing populations of the key pests in quinoa. Studies on the side effects of other active ingredients will also allow to select compatible insecticides with the biological control strategies in the context of an IPM approach.

Summary

Quinoa is an Andean grain with increasing popularity in the national and international market, due to its nutritional properties. In recent years, new quinoa production areas have emerged in South America, which include even coastal areas. In Peru, the lowlands have emerged as a new quinoa production zone, where there are better conditions to produce higher yields under a conventional system than in the highlands, the traditional quinoa production region, where organic quinoa is mostly cultivated. However, pest pressure appears to be higher in the coastal areas of Peru as compared with the Andean region (**Chapter 2**). In this dissertation, the insect diversity associated with quinoa in new production areas in Peru was evaluated and the thermal biology of two emerging pest from these areas, *Liorhyssus hyalinus* (Fabricius) (Hemiptera: Rhopalidae) and *Nysius simulans* (Hemiptera: Lygaeidae), was studied. Besides, selective and broad-spectrum insecticides against target species and their side effects on non-target species were assessed in order to be considered in IPM programs in quinoa. Moreover, the potential of the native lacewing *Chrysoperla externa* (Neuroptera: Chrysopidae) as a biocontrol agent of *L. hyalinus* and *N. simulans* was investigated.

Chapter 3 investigates the insect diversity associated with quinoa in three altitudinal zones of Peru by collecting insects with pitfall traps throughout the phenology of the crop. The field sites were San Lorenzo, belonging to a traditional quinoa production region in the Andean zone, Junín, and Majes and La Molina which belong to the non-traditional regions Arequipa and Lima, respectively. Our data revealed that the alpha diversity (in terms of species evenness and species richness) was highest in San Lorenzo, and lowest in Majes. As to the functional groups (herbivores and natural enemies), no differences between field sites in species evenness were found but San Lorenzo was significantly superior to the other zones in species richness of both functional guilds. The analysis of beta diversity revealed large differences among field sites in terms of entomofauna composition, with few species in common; the key pest of quinoa in South America, *Eurysacca melanocampta* (Lepidoptera: Gelechiidae), was found at the three localities.

Chapter 4 investigates the occurrence of insect pests and the natural enemies of quinoa in San Lorenzo (in the Andes), La Molina (at the coast) and Majes (in the “Maritime Yunga” ecoregion), by plant sampling and pitfall trapping. Our data indicated that the pest pressure in quinoa was higher at lower elevations than in the highlands. The major insect pest infesting quinoa at high densities in San Lorenzo was *E. melanocampta*; in La Molina, the major pests were *E.*

melanocampta, *Macrosiphum euphorbiae* (Hemiptera: Aphididae) and *Liriomyza huidobrensis* (Diptera: Agromyzidae); and in Majes, *Frankliniella occidentalis* (Thysanoptera: Thripidae) was the most abundant pest. The natural enemy complex was deemed to play an important role in controlling *M. euphorbiae* and *L. huidobrensis* by preventing pest resurgence.

Chapter 5 describes a field study which investigated the impact of four insecticides (cypermethrin, imidacloprid, teflubenzuron and emamectin benzoate) on insect pests of quinoa and their side effects on the arthropod community at the coastal level of Peru. We analysed the species composition and species diversity of arthropods, and the population density of phytophagous insects and their natural enemies. The arthropod community was examined with pitfall traps (for ground dwelling species), plant samplings (for pests and their natural enemies that inhabit the crop), and yellow pan traps (to catch flying insects). The results demonstrated that *M. euphorbiae*, *F. occidentalis* and *Spoladea recurvalis* (Lepidoptera: Crambidae) were efficiently controlled by cypermethrin and imidacloprid; the latter compound also showed long-term effects on the heteropteran pest *N. simulans*. Teflubenzuron and emamectin benzoate proved to be efficient to control *S. recurvalis*. Imidacloprid had the strongest adverse effects on the arthropod community in terms of species diversity, species composition and natural enemy density as compared with the other insecticides.

When quinoa is cultivated outside of its Andean origin, the heteropterans *L. hyalinus* and *N. simulans* may emerge as important pests in South America. In **Chapter 6** we studied the development and reproduction of both species at different constant temperatures in the laboratory. This information is essential not only to understand the life cycle of both species but also for pest risk analysis and integrated pest management. Egg and nymphal development were investigated at 18, 22, 26, 30, 34 and 36 °C; for both species, egg incubation time significantly decreased as the temperature increased, nymphs did not successfully develop at 18 °C and the total nymphal time significantly decreased as the temperature increased from 22 to 36 °C. Based on a linear day-degree (DD) model, the lower threshold temperatures for egg and nymphal development were estimated to be 16.0 and 18.1 °C for *L. hyalinus*, and 16.1 and 19.9 °C for *N. simulans*, respectively. Thermal requirements for egg and nymphal development were 68.6 and 111.2 DD for *L. hyalinus*, and 77.7 and 188.0 DD for *N. simulans*, respectively. The data indicate that more generations of *L. hyalinus* than of *N. simulans* through the year are expected. Reproduction and adult longevity were studied at 22, 26, 30 and 34 °C. For both species pre-oviposition time decreased as temperature increased, and the oviposition period was highest at 26 °C. The highest fecundity and egg viability were observed at 30 °C, whereas longevities were

higher at 22-26 °C than at 30-34 °C. Since the lowest tested temperatures were not suitable to both heteropterans and 30 °C was the optimal temperature for development and reproduction, high pest abundances in warm areas and seasons are expected.

In **Chapter 7**, the potential of the native lacewing *C. externa* as a biological control agent of *L. hyalinus* and *N. simulans* was evaluated by determining the predation capacity and development of the chrysopid larvae on immature stages of these heteropteran pests. In addition, prey preference was examined in two-prey combinations trials, i.e., in the absence or presence of *M. euphorbiae* as an alternative prey. Larvae of the predator were not able to feed on *L. hyalinus* eggs, but they effectively did on *N. simulans* eggs as well as on all nymphal instars of both species. Lacewing larvae became significantly more voracious with increasing instar, third instar larvae killing over three times the number of nymphal prey consumed by the second instar. Nymphs of *L. hyalinus* were less suitable prey for larval development of the predator than eggs of *Sitotroga cerealella* (Lepidoptera: Pyralidae), whereas *N. simulans* was overall an unsuitable prey. There was a clear prey preference of *C. externa* for aphids over the heteropterans, as well as a preference for *N. simulans* over *L. hyalinus*. Field experiments are needed to fully understand the potential of *C. externa* as a biological control agent of these heteropteran pests of quinoa.

In **Chapter 8**, a general discussion and future research perspectives are presented. Our study indicates that there are promising perspectives for implementing integrated control strategies both in the conventionally managed new production zones in the lowlands and in the traditional organic cultivation of the highlands of Peru. Our findings can contribute to a more sustainable production system for quinoa, not only in Peru and other South American countries, but also on other continents where quinoa is increasingly being cultivated. Further laboratory and field experiments are, however, needed to fully understand the efficiency of key natural enemies to help keep pest populations under non-injury levels, as well as studies to find selective insecticides which are compatible with biological controls.

Samenvatting

Quinoa is een pseudograan uit de Andes dat vanwege zijn nutritionele eigenschappen steeds populairder wordt op de nationale en internationale markt. De afgelopen jaren zijn er nieuwe productiegebieden van quinoa ontstaan in Zuid-Amerika, waaronder zelfs kustgebieden. In Peru zijn de laaglanden uitgegroeid tot een nieuwe productiezone, waar er betere omstandigheden zijn om op basis van een conventioneel systeem hogere opbrengsten te produceren dan in de hooglanden, de traditionele productieregio van quinoa, waar vooral biologisch wordt geteeld. De plaagdruk lijkt echter hoger te zijn in de kustgebieden van Peru dan in het Andesgebied (**Hoofdstuk 2**). In dit proefschrift werd de insectendiversiteit geassocieerd met quinoa in de nieuwe productiegebieden in Peru geëvalueerd. De thermische biologie van twee opkomende plagen uit deze gebieden, *Liorhyssus hyalinus* (Fabricius) (Hemiptera: Rhopalidae) en *Nysius simulans* (Hemiptera: Lygaeidae), werd in detail onderzocht. Ook werden selectieve en breedspectruminsecticiden tegen plaagsoorten en hun bijwerkingen op niet-doelsoorten beoordeeld om te worden overwogen in programma's van geïntegreerde bestrijding in quinoa. Bovendien werd het potentieel van de inheemse gaasvlieg *Chrysoperla externa* (Neuroptera: Chrysopidae) als biologische bestrijder van *L. hyalinus* en *N. simulans* bestudeerd.

Hoofdstuk 3 onderzoekt de insectendiversiteit geassocieerd met quinoa in drie hoogtezones van Peru door insecten te verzamelen met bodemvallen (pitfall traps) doorheen de fenologie van het gewas. De veldlocaties waren San Lorenzo, behorend tot een traditioneel quinoaproductiegebied in de Andes, Junín, en Majes en La Molina, die behoren tot respectievelijk de niet-traditionele regio's Arequipa en Lima. Uit onze gegevens bleek dat de alfadiversiteit (in termen van equitabiliteit en soortenrijkdom) het hoogst was in San Lorenzo en het laagst in Majes. Wat betreft de functionele groepen (herbivoren en natuurlijke vijanden), werden er geen verschillen gevonden tussen de locaties in termen van equitabiliteit, maar San Lorenzo was superieur aan de andere zones in soortenrijkdom van beide functionele gilden. De analyse van bèta-diversiteit bracht grote verschillen aan het licht tussen veldlocaties in termen van entomofauna-samenstelling, met weinig gemeenschappelijke soorten; de belangrijkste plaag van quinoa in Zuid-Amerika, *Eurysacca melanocampta* (Lepidoptera: Gelechiidae), werd gevonden op de drie locaties.

Hoofdstuk 4 onderzoekt het voorkomen van insectenplagen en hun natuurlijke vijanden in quinoa in San Lorenzo (in de Andes), La Molina (aan de kust) en Majes (in de "Maritime Yunga" ecoregio), door middel van directe bemonstering van planten en bodemvallen. Onze gegevens gaven aan dat de plaagdruk in quinoa hoger was op lagere hoogten dan in de hooglanden. De

belangrijkste insectenplaag die quinoa in hoge dichtheden in San Lorenzo teistert, was *E. melanocampta*; in La Molina waren de belangrijkste plagen *E. melanocampta*, *Macrosiphum euphorbiae* (Hemiptera: Aphididae) en *Liriomyza huidobrensis* (Diptera: Agromyzidae); in Majes was *Frankliniella occidentalis* (Thysanoptera: Thripidae) de meest voorkomende plaag. Het natuurlijke vijandcomplex werd geacht een belangrijke rol te spelen bij de bestrijding van *M. euphorbiae* en *L. huidobrensis* door de heropflakking van plagen te voorkomen.

Hoofdstuk 5 beschrijft een veldstudie waarin de impact van vier insecticiden (cypermethrine, imidacloprid, teflubenzuron en emamectinebenzoaat) op insectenplagen van quinoa en hun bijwerkingen op de geleedpotigengemeenschap in de kustzone van Peru is onderzocht. We analyseerden de soortensamenstelling en soortendiversiteit van de geleedpotigen, en de populatiedichtheid van fytofage insecten en hun natuurlijke vijanden. De geleedpotige gemeenschap werd onderzocht met bodemvallen (voor grondbewonende soorten), via directe plantenbemonstering (op herbivoren en hun natuurlijke vijanden in het gewas) en gele panvallen (voor het vangen van vliegende insecten). De resultaten toonden aan dat *M. euphorbiae*, *F. occidentalis* en *Spoladea recurvalis* (Lepidoptera: Crambidae) efficiënt werden bestreden door cypermethrine en imidacloprid; dit laatste insecticide vertoonde ook langetermijneffecten op de plaagwants *N. simulans*. Teflubenzuron en emamectinebenzoaat bleken efficiënt te zijn om *S. recurvalis* te bestrijden. Imidacloprid had de sterkste nadelige effecten op de geleedpotige gemeenschap in termen van soortendiversiteit, soortensamenstelling en de abundantie aan natuurlijke vijanden in vergelijking met de andere insecticiden.

Wanneer quinoa wordt geteeld in delen van Zuid-Amerika buiten de Andes, kunnen de wantsen *L. hyalinus* en *N. simulans* opduiken als belangrijke plagen. In **Hoofdstuk 6** hebben we de ontwikkeling en reproductie van beide soorten bij verschillende constante temperaturen in het laboratorium bestudeerd. Deze informatie is niet alleen essentieel om de levenscyclus van beide soorten te begrijpen, maar ook voor plaagrisicoanalyse en geïntegreerde bestrijding. De ontwikkeling van eitjes en nimfen werd onderzocht bij 18, 22, 26, 30, 34 en 36 °C. Voor beide soorten nam de incubatietijd van de eitjes significant af naarmate de temperatuur toenam, terwijl de nimfen zich niet succesvol ontwikkelden bij 18 °C en de totale nimfentijd significant afnam naarmate de temperatuur steeg van 22 tot 36 °C. Op basis van een lineair daggradenmodel (DD) werden de lagere drempeltemperaturen voor de ontwikkeling van eitjes en nimfen geschat op respectievelijk 16,0 en 18,1 °C voor *L. hyalinus* en 16,1 en 19,9 °C voor *N. simulans*. De warmtebehoefte voor de ontwikkeling van eitjes en nimfen was respectievelijk 68,6 en 111,2 DD voor *L. hyalinus* en 77,7 en 188,0 DD voor *N. simulans*. De data geven aan dat

er meer generaties van *L. hyalinus* dan van *N. simulans* door het jaar worden verwacht. Voortplanting en volwassen levensduur werden bestudeerd bij 22, 26, 30 en 34 °C. Voor beide soorten nam de pre-ovipositieperiode af naarmate de temperatuur toenam, en de ovipositieperiode was het langst bij 26 °C. De hoogste fecunditeit en levensvatbaarheid van de eitjes werden waargenomen bij 30 °C, terwijl de levensduur hoger was bij 22-26 °C dan bij 30-34 °C. Aangezien voor beide wantsen de laagste geteste temperaturen niet geschikt waren en 30 °C de optimale temperatuur was voor ontwikkeling en reproductie, worden hoge plaagpopulaties in warme gebieden en seizoenen verwacht.

In **Hoofdstuk 7** werd het potentieel van de inheemse gaasvlieg *C. externa* als biologische bestrijder van *L. hyalinus* en *N. simulans* geëvalueerd door het predatievermogen en de ontwikkeling van de gaasvlieglarven op onvolwassen stadia van deze plaagwantsen te bepalen. Bovendien werd de prooivoorkeur onderzocht in proeven met combinaties van twee prooien, met name in de aan- of afwezigheid van de bladluis *M. euphorbiae* als alternatieve prooi. Larven van de predator konden zich niet voeden met *L. hyalinus*-eitjes, maar wel met *N. simulans*-eitjes en met alle nimfenstadia van beide soorten. Gaasvlieglarven werden aanzienlijk vraatzuchtiger met toenemend stadium, waarbij larven in het derde stadium meer dan drie keer het aantal prooinimfen doodden dat door het tweede stadium werd geconsumeerd. Nimfen van *L. hyalinus* waren minder geschikte prooien voor de larvale ontwikkeling van *C. externa* dan eitjes van *Sitotroga cerealella* (Lepidoptera: Pyralidae), terwijl *N. simulans* over het algemeen een ongeschikte prooi bleek voor de ontwikkeling van de predator. Er was een duidelijke prooivoorkeur van de gaasvlieg voor bladluizen boven beide wantsen, evenals een voorkeur voor *N. simulans* boven *L. hyalinus*. Er zijn veldexperimenten nodig om het potentieel van *C. externa* als biologische bestrijder van deze wantsenplagen van quinoa volledig te kunnen inschatten.

In **hoofdstuk 8** worden een algemene discussie en toekomstige onderzoeksperspectieven gepresenteerd. Onze studie geeft aan dat er veelbelovende perspectieven zijn voor het implementeren van een geïntegreerde bestrijding, zowel in de conventioneel uitgebate nieuwe productiezones in de laaglanden als in de traditionele biologische teelt van de hooglanden van Peru. Onze bevindingen kunnen zo bijdragen aan een duurzamer productiesysteem voor quinoa, niet alleen in Peru en andere Zuid-Amerikaanse landen, maar ook op andere continenten waar steeds meer quinoa wordt verbouwd. Verdere laboratorium- en veldexperimenten zijn echter nodig om de efficiëntie van de belangrijkste natuurlijke vijanden om plaagpopulaties onder economische schadedrempels te houden volledig te kunnen begrijpen, evenals studies om

selectieve insecticiden te vinden die compatibel zijn met deze biologische bestrijdingsstrategieën.

References

- Ables, J. R., Jones, S. L., & McCommas, Jr. D. W. 1978. Response of selected predator species to different densities of *Aphis gossypii* and *Heliothis virescens* eggs. *Environmental Entomology*, 7: 402-404.
- Abugoch, L. E. 2009. Chapter 1 Quinoa (*Chenopodium quinoa* Willd.): Composition, chemistry, nutritional, and functional properties. *Advances in Food and Nutrition Research*, 58: 1–31.
- Alandia, G., Rodriguez, J. P., Jacobsen, S. E., Bazile, D., & Condori, B. 2020. Global expansion of quinoa and challenges for the Andean region. *Global Food Security*, 26: 100429.
- Alata-Cóndor, J. 1973. Lista de insectos y otros animales dañinos a la agricultura en el Perú. Estación Experimental Agrícola La Molina, Dirección General de Investigación Agraria.
- Albújar, E. 2017. Anuario estadístico de la producción agrícola 2017. Sistema Integrado de Estadísticas Agrarias del Ministerio de Agricultura y Riego del Perú. <http://siea.minagri.gob.pe/siea/?q=publicaciones/anuario-de-produccion-pecuaria>
- Albuquerque, G. S., Tauber, C. A., & Tauber, M. J. 1994. *Chrysoperla externa* (Neuroptera: Chrysopidae): Life history and potential for biological control in Central and South America. *Biological Control*, 4: 8-13.
- Albuquerque, G. S., Tauber, C. A., & Tauber, M. J. 2007. *Chrysoperla externa* and *Ceraeochrysa* spp.: Potential for biological control in the New World tropics and subtropics. In: McEwen, P., New, T., & Whittington, A. E. (Eds.). *Lacewings in the crop environment*. Cambridge University Press, New York, pp. 408–423.
- Altieri, M. 1999. The ecological role of biodiversity in agroecosystems. *Agriculture, Ecosystems & Environment*, 74: 19-31.
- Altieri, M., & Nicholls, C. 2004. *Biodiversity and pest management in agroecosystems*, second edition. Food Products Press, New York.
- Amjad, A., Azam, I., Sarwar, M. K., Malik, M. F., & Sattar, A. 2018. A review of imidacloprid toxicity in coccinellids. *Arthropods*, 7: 1-10.
- Amor, F., Medina, P., Bengochea, P., Canovas, M., Vega, P., Correia, R., García, F., Gómez, M., Budia, F., Viñuela, E., & López, J. A. 2012. Effect of emamectin benzoate under semi-field and field conditions on key predatory biological control agents used in vegetable greenhouses. *Biocontrol Science and Technology*, 22: 219-232.
- Angeli, V., Miguel Silva, P., Crispim Massuela, D., Waleed-Khan, M., Hamar, A., Khajehei, F., Graeff-Hönninger, S., Piatti, C. 2020. Quinoa (*Chenopodium quinoa* Willd.): An overview of the potentials of the “golden grain” and socio-economic and environmental aspects of its cultivation and marketization. *Foods*, 9: 216.
- Angulo, A. O., Olivares, T. S., & Weigert, G. T. 2006. Estados inmaduros de Lepidópteros noctuidos de importancia económica agrícola y forestal en Chile (Lepidoptera: Noctuidae). Impresos Siglo Veintiuno Ltda, Concepción, Chile.

- Angulo, A. O., Olivares, T. S., & Weigert, G. T. 2008. Estados inmaduros de lepidópteros noctuidos de importancia agrícola y forestal en Chile y claves para su identificación (Lepidoptera: Noctuidae), third edition. Universidad de Concepción, Chile.
- Antezana-Febres, E., Ibáñez-Tremolada, M., García, Y., & Pando, L. 2019. Tolerancia de la quinua (*Chenopodium quinoa* Willd.) al efecto combinado sequía—calor en siembras de verano en la costa central del Perú. VII Congreso Mundial de la Quinua y Otros Granos Andinos, Iquique, Chile.
- Arenas, L. 2019. Determinación del ciclo de vida de *Liorhyssus hyalinus* (F.) en condiciones de laboratorio y bajo temperaturas controladas en la Irrigación Majes [Bachelor thesis]. Universidad Católica de Santa María, Arequipa, Peru.
- Aragón, J., & Flores, F. 2006. Control integrado de plagas en soja en el sudeste de Córdoba. Instituto Nacional de Tecnología Agropecuaria. https://inta.gob.ar/sites/default/files/script-tmp-control_integrado_de_plagas_en_soja_en_el_sudeste_de_.pdf
- Arnett, Jr. R., Frank, J., Thomas, M., & Skelley, P. 2002. American beetles: Polyphaga: Scarabaeoidea through Curculionoidea (Vol. 2). CRC Press, New York.
- Arnett, Jr. R., & Thomas, M. 2000. *American beetles: Archostemata, Myxophaga, Adepaga, Polyphaga: Staphyliniformia* (Vol. 1). CRC Press, New York.
- Bahar, M. H., Stanley, J., Gregg, P., & Socorro, A. D. 2013. Predation of cotton bollworm by green lacewings in the presence of cotton aphid as alternative prey on transgenic *Bt* and conventional cotton. *Entomologia Experimentalis et Applicata*, 146: 224-231.
- Baker, B. P., Green, T. A., & Loker, A. J. 2020. Biological control and integrated pest management in organic and conventional systems. *Biological Control*, 140: 104095.
- Bale, J., Harrington, R., & Clough, M. 1988. Low temperature mortality of the peach-potato aphid *Myzus persicae*. *Ecological Entomology*, 13: 121-129.
- Barzman, M., Bàrberi, P., Birch, A. N., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J. E., Kiss, J., Kudsk, P., Lamichhane, J. R., Messéan, A., Moonen, A., Ratnadass, A., Ricci, P., Sarah, J. L., Sattin, M. 2015. Eight principles of integrated pest management. *Agronomy for Sustainable Development*, 35: 1199-1215.
- Basantes-Morales, E. R., Alconada, M. M., & Pantoja, J. L. 2019. Quinoa (*Chenopodium quinoa* Willd) Production in the Andean Region: Challenges and Potentials. *Journal of Experimental Agriculture International*, 36: 1-18.
- Bastidas, J. S., Devia, E. H., & Amaya, O. S. 2010. Cría y evaluación de la capacidad de depredación de *Chrysoperla externa* sobre *Neohydatothrips signifer*, trips plaga del cultivo de maracuyá. *Ciencia & Tecnología Agropecuaria*, 11: 31-40.
- Bayoumy, A. T., Mohamed, A. H., Hussein, M. I., Tantawy, M. E., & Salim, M. A. 2020. Pollen criteria as a taxonomic tool to clarify the relationships between some taxa of Chenopodiaceae and Amaranthaceae. *The Egyptian Journal of Experimental Biology (Botany)*, 16: 49-58.
- Bazile, D., & Baudron, F. 2014. Dinámica de expansión mundial del cultivo de la quinua respecto a su alta biodiversidad. In: Bazile, D., Bertero, D., Nieto, C. (Eds.). Estado del arte de la quinua en el mundo en 2013. FAO (Santiago de Chile) y CIRAD (Montpellier, Francia), pp. 49–64. <http://www.fao.org/3/a-i4042e.pdf>

- Bazile, D., Bertero, D., Nieto, C. 2014. Estado del arte de la quinua en el mundo en 2013. FAO (Santiago de Chile) y CIRAD (Montpellier, Francia).
- Bazile, D., Jacobsen, S. E., & Verniau, A. 2016. The global expansion of quinoa: Trends and limits. *Frontiers in Plant Science*, 7: 622.
- Bedoya-Perales, N., Pumi, G., Mujica, A., Talamini, E., & Domingos Padula, A. 2018a. Quinoa expansion in Peru and its implications for land use management. *Sustainability*, 10: 532.
- Bedoya-Perales, N., Pumi, G., Talamini, E., & Domingos Padula, A. 2018b. The quinoa boom in Peru: Will land competition threaten sustainability in one of the cradles of agriculture? *Land Use Policy*, 79: 475-480.
- Begg, G. S., Cook, S. M., Dye, R., Ferrante, M., Franck, P., Lavigne, C., Lövei, G. L., Mansion-Vaquie, a., Pell, J. K., Petit, S., Quesada, N., Ricci, B., Wratten, S. D., & Birch, A. N. E. 2017. A functional overview of conservation biological control. *Crop Protection*, 97: 145-158.
- Bennett, A. 2010. The role of soil community biodiversity in insect biodiversity. *Insect Conservation and Diversity*, 3: 157–171.
- Bentancourt, C. M., & Scatoni, I. B. 2006. Lepidópteros de importancia económica en el Uruguay: Reconocimiento, biología y daños de las plagas agrícolas y forestales, second edition. Hemisferio Sur, Montevideo, Uruguay.
- Bezerra, C. E. S., Amaral, B. B., & Souza, B. 2017. Rearing *Chrysoperla externa* larvae on artificial diets. *Neotropical Entomology*, 46: 93-99.
- Biondi, M., & D’Alessandro, P. 2012. Afrotropical flea beetle genera: A key to their identification, updated catalogue and biogeographical analysis (Coleoptera, Chrysomelidae, Galerucinae, Alticini). *Zookeys*, 253: 1-158.
- Blackman, R., & Eastop, V. 2000. Aphids on the world’s crops: An identification and information guide, second edition, Vols 1, 2. John Wiley & Sons Ltd, Chichester.
- Blackman, R., & Eastop, V. 2006. Aphids on the world’s herbaceous plants and shrubs: Vol. 1,2. John Wiley & Sons, Chichester.
- Blackman, R., & Eastop, V. F. 2017. Taxonomic issues. In: Van Emden, H. F., & Harrington, R. (Eds.). Aphids as crop pests. CAB International, Trowbridge, pp. 1–29.
- Blanco, A. 1994. Umbral económico de kcona kcona, *Eurysacca melanocampta* (Lepidoptera Gelechiidae) en quinua (*Chenopodium quinoa* Willd.) [Bachelor thesis]. Universidad Nacional del Altiplano, Puno, Peru.
- Bonte, J. 2016. Ecology and biocontrol potential of the South African flower bugs *Orius thripoborus* and *Orius naivashae* [Doctoral dissertation]. Ghent University, Ghent, Belgium.
- Bonte, J., De Hauwere, L., Conlong, D., & De Clercq, P. 2015. Predation capacity, development and reproduction of the southern African flower bugs *Orius thripoborus* and *Orius naivashae* (Hemiptera: Anthocoridae) on various prey. *Biological Control*, 86: 52-59.
- Bonte, J., De Ro, M., Conlong, D., & De Clercq, P. 2012. Thermal biology of the predatory bugs *Orius thripoborus* and *O. naivashae* (Hemiptera: Anthocoridae). *Environmental Entomology*, 41: 989-996.

- Borsdorf, A., & Stadel, C. 2015. The Andes: A geographical portrait. Springer Geography, New York.
- Bouček, Z., & Rasplus, J. 1991. Illustrated key to West-Palearctic genera of Pteromalidae (Hymenoptera: Chalcidoidea). Institut National de la Recherche Agronomique (INRA), Paris.
- Bousquet, Y. 2010. Illustrated identification guide to adults and larvae of Northeastern North American ground beetles (Coleoptera, Carabidae). Sofia-Moscow: Pensoft, Bulgaria.
- Brier, H. 2007. Pulses-Summer (including peanuts). In: Bailey, P. T. (Ed.). Pests of field crops and pastures: Identification and control. CSIRO Publishing, Malaysia, pp. 169–257.
- Brown, B., Borkent, A., Cumming, J., Wood, D., Woodley, N., & Zumbado, M. 2010. Manual of Central American Diptera (Vol. 2). NRC Research Press, Ottawa, Ontario, Canada.
- Brown, B., Borkent, A., Cumming, J., Wood, D., & Zumbado, M. 2009. Manual of Central American Diptera (Vol. 1). NRC Research Press, Ottawa, Ontario, Canada.
- Brown, M. W., & Adler, C. R. 1989. Community structure of phytophagous arthropods on apple. *Environmental Entomology*, 18: 600–607.
- Burckhardt, D. 1988. Jumping plant lice (Homoptera: Psylloidea) of the temperate neotropical region. Part 3: Calophyidae and Triozidae. *Zoological Journal of the Linnean Society*, 92: 115-191.
- Burckhardt, D. 1994. Generic key to Chilean jumping plant-lice (Homoptera: Psylloidea) with inclusion of potential exotic pests. *Revista Chilena de Entomología*, 21: 57-67.
- Burckhardt, D. 1987a. Jumping plant lice (Homoptera: Psylloidea) of the temperate neotropical region. Part 1: Psyllidae (subfamilies Aphalarinae, Rhinocolinae and Aphalaroidinae). *Zoological Journal of the Linnean Society*, 89: 299-392.
- Burckhardt, D. 1987b. Jumping plant lice (Homoptera: Psylloidea) of the temperate neotropical region. Part 2: Psyllidae (subfamilies Diaphorininae, Acizziinae, Ciriacreminae and Psyllinae). *Zoological Journal of the Linnean Society*, 90: 145-205.
- Burgos, A. 2013. Efecto de la temperatura en la biología y comportamiento de *Diglyphus websteri* (Crawford) (Hymenoptera: Eulophidae) [Master thesis]. Universidad Nacional Agraria La Molina, Lima, Peru.
- Caballero, R., Habeck, D. H., & Andrews, K. L. 1994. Clave ilustrada para larvas de Noctúidos de importancia económica de El Salvador, Honduras y Nicaragua. *Ceiba*, 35: 225-237.
- CABI. 2020. Invasive species compendium, *Spoladea recurvalis* (Hawaiian beet webworm). Wallingford, UK: CAB International. <https://www.cabi.org/isc/datasheet/28245>
- CABI. 2021. Invasive species compendium, *Frankliniella occidentalis* (western flower thrips). Wallingford, UK: CAB International. <https://www.cabi.org/isc/datasheet/24426>
- Cáceres del Carpio, F. A., & Iannacone, J. 2021. Evaluación del riesgo ambiental por los insecticidas fipronil e imidacloprid en el camarón de río (*Cryphiops caementarius*). La granja. *Revista de Ciencias de La Vida*, 33: 104-114.
- Callohuari, Y., Vergara, C., & Jiménez, J. 2018. Insect pests associated with Andean lupin (*Lupinus mutabilis* Sweet) and their parasitoids in Peruvian central coast (Lima, La Molina). *Peruvian Journal of Agronomy*, 2: 27-33.

- Calvo-Agudo, M., González-Cabrera, J., Picó, Y., Calatayud-Vernich, P., Urbaneja, A., Dicke, M., & Tena, A. 2019. Neonicotinoids in excretion product of phloem-feeding insects kill beneficial insects. *Proceedings of the National Academy of Sciences*, 116: 16817-16822.
- Camino, A., & Johns, T. 1988. Laki-Laki (*Dennstaedtia glauca*, Polypodiaceae): A green manure used in traditional Andean agriculture. *Economic Botany*, 42: 45-53.
- Camino, A., Recharte, J., & Bidegaray, P. 1985. Calendar flexibility in traditional agriculture of the Eastern slopes of the Andes. In: Lechtman, H., & Soldi, A. M. (Eds.). *La tecnología en el mundo andino*. Universidad Nacional Autónoma de México Press, Ciudad de Mexico, pp. 169-194.
- Campbell, A., Frazer, B., Gilbert, N., Gutierrez, A., & Mackauer, M. 1974. Temperature requirements of some aphids and their parasites. *Journal of applied ecology. Journal of Applied Ecology*, 11: 431-438.
- Campos, D., & Sharkey, M. 2006. Familia Braconidae. In: Fernández, F., & Sharkey, M. (Eds.). *Introducción a los Hymenoptera de La Región Neotropical*. Sociedad Colombiana de Entomología y Universidad Nacional de Colombia, Bogotá, Colombia, pp. 3313-3365.
- Canard, M. 2007. Natural food and feeding habits of lacewings. In: McEwen, P., New, T., & Whittington, A. E. (Eds.). *Lacewings in the crop environment*. Cambridge University Press, New York, pp. 116-129.
- Cancino-Espinoza, E., Vázquez-Rowe, I., & Quispe, I., & Quispe, I. 2018. Organic quinoa (*Chenopodium quinoa* L.) production in Peru: Environmental hotspots and food security considerations using life cycle assessment. *Science of the Total Environment*, 637: 221-232.
- Capinera, J. 2020. *Handbook of vegetable pests*, second edition. Academic press, London, San Diego.
- Cardoso, J. T., & Lazzari, S. 2003. Development and consumption capacity of *Chrysoperla externa* (Hagen) (Neuroptera, Chrysopidae) fed with *Cinara* spp. (Hemiptera, Aphididae) under three temperatures. *Revista Brasileira de Zoologia*, 20: 573-576.
- Carrasco, F. 1987. Insectos en la «kiwicha» cultivada en Cusco y Apurímac. *Revista Peruana de Entomología*, 30: 38-41.
- Carrera, C. 2013. Ciclo biológico y morfología de *Copitarsia corruda* Pogue & Simmons, *Heliothis virescens* (Fabricius), *Spodoptera frugiperda* (J. E. Smith) y *Spodoptera ochrea* (Hampson), en turiones de espárrago [Master thesis]. Universidad Nacional Agraria La Molina, Lima, Peru.
- Carrera, C., Cruces, L., & Callohuari, Y. 2016. Insectos masticadores de granos de la panoja. In: Cruces, L., Callohuari, Y., & Carrera, C. (Eds.). *Quinua manejo integrado de plagas. Estrategias en el cultivo de la quinua para fortalecer el sistema agroalimentario en la zona andina*. Organización de las Naciones Unidas para la Alimentación y la Agricultura, Santiago, Chile, pp. 29-45.
- Carvalho, C. F., & Souza, B. 2000. Métodos de criação e produção de crisopídeos. In: Bueno V. H. (Ed.). *Controle biológico de pragas: Produção massal e controle de qualidade*. Universidade Federal de Lavras, Minas Gerais, Brazil, pp. 91-103.
- Carver, M., & Franzmann, B. 2001. *Lysiphlebus* Foerster (Hymenoptera: Braconidae: Aphidiinae) in Australia. *Australian Journal of Entomology*, 40: 198-201.

- Castle, S. J. 1999. Agricultural intensification and pest outbreaks: A reappraisal of events in the Sudan Gezira. *Annals of the Entomological Society of America*, 92: 840-852.
- Castro, V., & Araya, J. 2012. Clave de identificación de huevos, larvas y pupas de *Allograpta* (Diptera: Syrphidae) comunes en la zona central de Chile. *Boletín de Sanidad Vegetal. Plagas*, 38: 83-94.
- Catalogue of Life. 2022. Web site: <https://www.catalogueoflife.org/>
- Cermeli, M., Sánchez, J., Morales, P., & Godoy, F. 2004. *Liorhyssus hyalinus* (F.) (Hemiptera: Rhopalidae) nueva plaga del sorgo en Venezuela. *Entomotropica*, 19: 101-103.
- Chacón-Galindo, C. 1963. *Gnorimoschema* sp. (Gelechiidae - Lepidoptera) en Quinoa. *Revista Peruana de Entomología*, 6: 15-20.
- Chakraborty, S., & Chatterjee, M. L. 1999. Effect of four benzophenylureas on population of safflower aphid, *Dactynotus carthami* HRL. and lady bird predators, *Coccinella septempunctata* L. and *Coccinella* sp. *Indian Journal of Experimental Biology*, 37: 374-378.
- Chazdon, R., Colwell, R., Denslow, J., & Guariguata, M. 1998. Statistical methods for estimating species richness of woody regeneration in primary and secondary rain forests of northeastern Costa Rica. In: Dallmeier, F., & Comiskey, J. A. (Eds.). *Forest biodiversity research, monitoring and modeling: Conceptual background and old world case studies*. Man and the Biosphere Series, USA, pp. 285–309.
- Childers, C. C., & Achor, D. S. 1995. Thrips feeding and oviposition injuries to economic plants, subsequent damage and host responses to infestation. In: Parker, B.L., Skinner, M., & Lewis, T. (Eds.). *NATO ASI Series (Series A: Life Sciences)*, vol 276. Springer, Boston, MA, pp. 31–51.
- Chorbadjian, R. A., Ahumada, M. I., Urra, F., Elgueta, M., & Gilligan, T. M. 2021. Biogeographical patterns of herbivore arthropods associated with *Chenopodium quinoa* grown along the latitudinal gradient of Chile. *Plants*, 10: 2811.
- Chown, S. L., & Nicolson, S. 2004. *Insect physiological ecology: Mechanisms and patterns*. Oxford University Press, Oxford.
- Cisneros, F. 2012. *Control químico de las plagas agrícolas*. Sociedad Entomológica del Perú, Lima, Peru.
- Clarke-Harris, D., Fleischer, S. J., Fuller, C., & Bolton, J. 2004. Evaluation of the efficacy of new chemistries for controlling major lepidoptera pests on vegetable amaranth in Jamaica. *CARDI Review*, 4: 12-19.
- Cloyd, R. A. 2020. How effective is conservation biological control in regulating insect pest populations in organic crop production systems? *Insects*, 11: 744.
- Cohen-Aponte. 2019. Introduction to ancient Andean art. Smarthistory. <https://smarthistory.org/introduction-to-ancient-andean-art/>
- Colwell, R. 2013. *EstimateS* (9.1.0) [Computer software]. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, USA.
- Corder, G., & Foreman, D. 2009. *Nonparametric statistics for non-statisticians: A step-by-step approach* (Vol. 1). John Wiley & Sons.

- Cornelis, M. 2015. Biodiversidad de Nabidae (Insecta: Heteroptera): Revisión taxonómica y análisis cladístico del género *Nabis* Latreille, 1802. [Doctoral dissertation] Universidad Nacional de La Plata, Argentina.
- Cornelis, M., & Coscarón, M. 2013. The Nabidae (Insecta, Hemiptera, Heteroptera) of Argentina. *ZooKeys*, 333: 1-30.
- Cornelis, M., Quiran, E., & Coscaron, M. 2012. The scentless plant bug, *Liorhyssus hyalinus* (Fabricius) (Hemiptera: Heteroptera: Rhopalidae): Description of immature stages and notes on its life history. *Zootaxa*, 3525: 83-88.
- Costa, J., Cosio, W., Cardenas Molina, M., Yábar, E., & Gianoli, E. 2009a. Preference of quinoa moth *Eurysacca melanocampta* Meryck (Lepidoptera: Gelechiidae) for two varieties of quinoa (*Chenopodium quinoa* Willd) in olfactometry assays. *Chilean Journal of Agricultural Research*, 69: 71–78.
- Costa, J., Yábar, E., & Gianoli, E. 2009b. Parasitism on *Eurysacca melanocampta* Meyrick (Lepidoptera: Gelechiidae) in two localities at Cusco, Peru. *Revista Facultad Nacional de Agronomía, Medellín*, 62: 4807-4813.
- Costa-Lima, A. 1940. *Insetos do Brasil*. Tomo 2, Hemípteros. Escola Nacional de Agronomia, 351 pp.
- Cranshaw, W., Kondratieff, B., & Qian, T. 1990. Insects associated with quinoa, *Chenopodium quinoa*, in Colorado. *Journal of the Kansas Entomological Society*, 63: 195-199.
- Crespo, L., & Saravia, R. 2014. Insectos plaga ocasionales en el cultivo de quinua. In: Saravia, R., Plata, G., & Gandarillas, A. (Eds.). *Plagas y enfermedades del cultivo de quinua*. Fundación PROINPA, Cochabamba, Bolivia, pp. 63–81.
- Croft, B., & Whalon, M. 1982. Selective toxicity of pyrethroid insecticides to arthropod natural enemies and pests of agricultural crops. *Entomophaga*, 27: 3-21.
- Crowder, D. W., & Jabbour, R. 2014. Relationships between biodiversity and biological control in agroecosystems: Current status and future challenges. *Biological Control*, 75: 8–17.
- Cruces, L., Callohuari, Y., & Carrera, C. 2016. Quinua: Manejo integrado de plagas. Estrategias en el cultivo de quinua para fortalecer el sistema agroalimentario en la zona andina. Organización de las naciones unidas para la alimentación y la agricultura, Santiago, Chile.
- Cruces, L., de la Peña, E., & De Clercq, P. 2020a. Insect diversity associated with quinoa (*Chenopodium quinoa* Willd.) in three altitudinal production zones of Peru. *International Journal of Tropical Insect Science*, 40, 955-968.
- Cruces, L., de la Peña, E., & De Clercq, P. 2020b. Seasonal phenology of the major insect pests of quinoa (*Chenopodium quinoa* Willd.) and their natural enemies in a traditional zone and two new production zones of Peru. *Agriculture*, 10: 644.
- Cruces, L., de la Peña, E., & De Clercq, P. 2021. Field evaluation of cypermethrin, imidacloprid, teflubenzuron and emamectin benzoate against pests of quinoa (*Chenopodium quinoa* Willd.) and their side effects on non-target species. *Plants*, 10: 1788.
- Crumb, S. E. 1956. The larvae of the Phalaenidae. *USDA. Technical Bulletin* N° 1135.

- Cruz, A. 2017. Situación actual del consumo de pesticidas en el Perú [Bachelor thesis]. Universidad Nacional Agraria La Molina, Lima, Peru.
- Cuello, E. M., Andorno, A. V., Hernandez, C. M., & Lopez, S. N. 2019. Prey consumption and development of the indigenous lacewing *Chrysoperla externa* feeding on two exotic *Eucalyptus* pests. *Biocontrol Science and Technology*, 29: 1159-1171.
- Dadther-Huaman, H., Machaca-Paccara, A., & Quispe-Castro, R. 2020. Eficacia de nueve métodos de control de *Oregmomyza peruviana* (Granara de Willink & Diaz) (Hemiptera: Coccoidea: Eriococcidae) en *Vitis vinifera* L. 'Negra Criolla' y 'Quebranta'. *Scientia Agropecuaria*, 11: 95-103.
- Dalazen, G., Guedes, J. V. C., Carpintero, D. L., Stacke, R. F., & Cagliari, D. 2014. Populational fluctuation of *Nysius simulans* associated with soybean and hairy fleabane in Brazil. *Interciencia*, 39: 391-394.
- De Clercq, P., & Degheele, D. 1992. Development and survival of *Podisus maculiventris* (Say) and *Podisus sagitta* (Fab.) (Heteroptera: Pentatomidae) at various constant temperatures. *The Canadian Entomologist*, 124: 125-133.
- De Conti, B. F., Castro, V. P., Sampaio, M. V., & van Lenteren, J. C. 2011. Development and survival of *Aulacorthum solani*, *Macrosiphum euphorbiae* and *Uroleucon ambrosiae* at six temperatures. *Bulletin of Insectology*, 64: 63-68.
- De la Cruz, L., Silva, D., & Vergara, C. 2019. Composición y fluctuación poblacional de la araneofauna en el algodónero de la Universidad Nacional Agraria La Molina, Lima, Perú. *Revista peruana de biología*, 26: 63-80.
- De Mendiburu, F. 2020. Agricolae: Statistical procedures for agricultural research. R package version, 1.3(3). <https://cran.r-project.org/web/packages/agricolae/agricolae.pdf>
- Delbeke, F., Vercruyse, P., Tirry, L., De Clercq, P., & Degheele, D. 1997. Toxicity of diflubenzuron, pyriproxyfen, imidacloprid and diafenthiuron to the predatory bug *Orius laevigatus* (Het.: Anthocoridae). *Entomophaga*, 42, 349–358.
- Delgado-Zegarra, J., Alvarez-Risco, A., & Yáñez, J. A. 2018. Uso indiscriminado de pesticidas y ausencia de control sanitario para el mercado interno en Perú. *Revista Panamericana de Salud Pública*, 42:e3.
- Derocles, S. A., Le Ralec, A., Plantegenest, M., Chaubet, B., Cruaud, C., Cruaud, A., & Rasplus, J. 2012. Identification of molecular markers for DNA barcoding in the Aphidiinae (Hym. Braconidae). *Molecular Ecology Resources*, 12:197–208.
- Desneux, N., Decourtye, A., & Delpuech, J. M. 2007. The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, 52: 81–106.
- Devine, G. J., Harling, Z. K., Scarr, A. W., & Devonshire, A. L. 1996. Lethal and sublethal effects of imidacloprid on nicotine-tolerant *Myzus nicotianae* and *Myzus persicae*. *Pesticide Science*, 48:57-62.
- Ding, T., Chi, H., Gökçe, A., Montoro, Y., & Zhang, B. 2018. Demographic analysis of arrhenotokous parthenogenesis and bisexual reproduction of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Scientific Reports*, 8: 3346.

- Ding, Y., & Chen, Y. P. 1986. Predation pattern of *Chrysoperla (Chrysopa) sinica* on cotton aphid and cotton bollworm. *Chinese Journal of Biological Control (China)*, 2: 97–102.
- Di Iorio, O. 2004. Hemíptera: Lygaeidae. In: Cordo, H. A., Logarzo, G., Brown K., & Di Iorio, O. (Eds.). Catálogo de insectos fitófagos de la Argentina y sus plantas asociadas. Sociedad Entomológica Argentina Ediciones, Buenos Aires, pp. 249-253.
- Dos Santos, K. B., Meneguín, A. M., Dos Santos, W. J., Neves, P. M., & Dos Santos, R. B. 2010. Caracterização dos danos de *Spodoptera eridania* (Cramer) e *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae) a estruturas de algodoeiro. *Neotropical Entomology*, 39: 626-631.
- Douglas, M. R., & Tooker, J. F. 2016. Meta-analysis reveals that seed-applied neonicotinoids and pyrethroids have similar negative effects on abundance of arthropod natural enemies. *PeerJ*, 4: e2776.
- Drescher, W., & Geusen-Pfister, H. 1991. Comparative testing of the oral toxicity of acephate, dimethoate and methomyl to honeybees, bumblebees and Syrphidae. *Acta Horticulturae*, 288: 133-138.
- Du Plessis, H., Van den Berg, J., & Byrne, M. J. 2011. The effect of temperature on *Nysius natalensis* Evans (Hemiptera: Orsillidae) development and survival. *African Entomology*, 19: 709-716.
- Dughetti, A. C. 2015b. La chinche diminuta *Nysius simulans*: Plaga emergente en quinua y otros cultivos en el valle bonaerense del Río Colorado. Departamento de Agronomía, Universidad Nacional del Sur. http://www.servicios.uns.edu.ar/institucion/files/1_AP_0_68.pdf
- Dughetti, A. C. 2015a. Plagas de la Quinua y sus enemigos naturales en el Valle inferior del río Colorado, Buenos Aires, Argentina. Estación Experimental Hilario Ascasubi, INTA Edditions. Buenos Aires. <https://inta.gob.ar/sites/default/files/script-tmp-inta-manual-plagas-de-la-quinua-y-sus-enemigos-natura.pdf>
- Dughetti, A. C., Carpintero, D., Navarro, F., La Rossa, F., Aquino, D., Martínez, J. J., & Zárate, A. 2013. Artrópodos presentes en la quinua en el valle inferior del Río Colorado, Buenos Aires, Argentina. *Ciencia y Tecnología de Los Cultivos Industriales. Ediciones INTA*, 3: 45-52.
- Echevarría, A., Gimeno, C., & Jimenez, R. 1994. *Liriomyza huidobrensis* (Blanchard, 1926) (Diptera, Agromyzidae) a new pest on crops in Valencia. *Boletín de Sanidad Vegetal. Plagas (España)*, 20: 103-109.
- Eiselen, E. 1956. Quinoa, a potentially important food crop of the Andes. *Journal of Geography*, 55: 330–333.
- El-Arnaouty, S. A., Ferran, A., & Beyssat-Arnaouty, V. 1996. Food consumption by *Chrysoperla carnea* (Stephens) and *Chrysoperla sinica* (Tjeder) of natural and substitute prey: Determination of feeding efficiency (Insecta: Neuroptera: Chrysopidae). International Symposium on Neuropterology (5th: 1994: Cairo, Egypt). Pure and applied research in neuropterology, Toulouse, France.
- Elbert, A., Nauen, R., & Leicht, W. 1998. Imidacloprid, a novel chloronicotinyl insecticide: Biological activity and agricultural importance. In: Ishaaya, I., & Degheele, D. (Eds.). Insecticides with novel modes of action: Mechanisms and application. Springer, Berlin, Germany, pp. 50–73.

- El-Naggar, J. B., & Zidan, N. E. 2013. Field evaluation of imidacloprid and thiamethoxam against sucking insects and their side effects on soil fauna. *Journal of Plant Protection Research*, 53: 375-387.
- El-Wakeil, N., Gaafar, N., Sallam, A., & Volkmar, C. 2013. Side effects of insecticides on natural enemies and possibility of their integration in plant protection strategies. In: Trdan, S. (Ed.). *Insecticides-development of safer and more effective technologies*. InTech, Rijeka, Croatia, pp. 4–56.
- Epstein, D., Zack, R., Brunner, J., Gut, L., & Brown, J. 2000. Effects of broad-spectrum insecticides on epigeal arthropod biodiversity in Pacific Northwest apple orchards. *Environmental Entomology*, 29: 340-348.
- Eshete, M. A., Asfaw, Z., & Kelbessa, E. 2016. A review on taxonomic and use diversity of the family Amaranthaceae in Ethiopia. *Journal of Medicinal Plants Sciences*, 4: 185-194.
- European Food Safety Authority. 2012. Conclusion on the peer review of the pesticide risk assessment of the active substance emamectin. *EFSA Journal*, 10: 2955.
- Fairtrade, 2022. Website: <https://www.fairtrade.net/>
- FAO. 2006. Guidelines on efficacy evaluation for the registration of plant protection products. FAO Publications, Rome, Italy.
- FAO. 2011. Quinoa: An ancient crop to contribute to world food security. Food and Agriculture Organization, Regional Office for Latin America and the Caribbean.
- FAO. 2022. FAOSTAT. In: Data—Crops: Quinoa for Bolivia, Peru, Ecuador, Yield, Area Harvested, Production Quantity for 2000—2020. Food and Agriculture Organization of the United Nations. <http://www.fao.org/faostat/en/#data/QC>
- FAO, & Bioersivity international. 2012. *Celebrando el Año Internacional de la Quinoa: Un futuro sembrado hace miles de años*. http://www.fao.org/fileadmin/templates/aiq2013/res/es/nota_conceptual.pdf
- Fernández, F., & Sharkey, M. 2006. Introducción a los Hymenoptera de la Región Neotropical. Sociedad Colombiana de Entomología y Universidad Nacional de Colombia, Bogotá, Colombia.
- Forister, M. L., Pelton, E. M., & Black, S. H. 2019. Declines in insect abundance and diversity: We know enough to act now. *Conservation Science and Practice*, 1: e80.
- Fowles, T. M., Coscarón, M. D., Panizzi, A. R., & Carroll, S. P. 2015. Scentless Plant Bugs (Rhopalidae). In: Panizzi, A., & Grazia, J. (Eds.). *True bugs (Heteroptera) of the Neotropics*. Springer, pp. 607–637.
- Fonseca, A. R., Carvalho, C. F., Cruz, I., Souza, B., & Ecole, C. C. 2015. Development and predatory capacity of *Chrysoperla externa* (Neuroptera: Chrysopidae) larvae at different temperatures. *Revista Colombiana de Entomología*, 41: 5-11.
- Frison, E. A., Cherfas, J., & Hodgkin, T. 2011. Agricultural biodiversity is essential for a sustainable improvement in food and nutrition security. *Sustainability*, 3: 238-253.
- Froeschner, R. C. 1981. Heteroptera or true bugs of Ecuador: A partial catalog. *Smithsonian Contributions to Zoology*.

- Funderburk, J., Srivastava, M., Funderburk, C., & McManus, S. 2013. Evaluation of imidacloprid and cyantraniliprole for suitability in conservation biological control program for *Orius insidiosus* (Hemiptera: Anthocoridae) in field pepper. *The Florida Entomologist*, 96: 229-231.
- Fye, R. E., & McAda, W. C. 1972. Laboratory studies on the development, longevity, and fecundity of six lepidopterous pests of cotton in Arizona. *USDA Technical Bulletin* 1454.
- Gamboa, C., Van den Broeck, G., & Maertens, M. 2018. Smallholders' preferences for improved quinoa varieties in the Peruvian Andes. *Sustainability*, 10: 3735.
- Gamboa, S., Souza, B., & Morales, R. 2016. Predatory activity of *Chrysoperla externa* (Neuroptera: Chrysopidae) on *Macrosiphum euphorbiae* (Hemiptera: Aphididae) in *Rosa* sp. *Crop. Revista Colombiana de Entomología*, 42: 54-58.
- Gandarillas, A., Rojas, W., Bonifacio, A., & Ojeda, N. 2014. La quinua en Bolivia: Perspectiva de la Fundación PROINPA. In: Bazile, D., Bertero, D., Nieto, C. (Eds.). Estado del arte de la quinua en el mundo en 2013. FAO (Santiago de Chile) y CIRAD, (Montpellier, Francia), pp. 410–431.
- García, M., Condori, B., & Castillo, C. 2015. Agroecological and agronomic cultural practices of quinoa in South America. In: Murphy, K. S., & Matanguihan, J. (Eds.). Quinoa: Improvement and sustainable production. John Wiley & Sons, Inc. pp. 25–46.
- Garzón, A., Freire, B. C., Carvalho, G. A., Oliveira, R. L., Medina, P., & Budia, F. 2015. Development and reproduction of *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) fed on *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) vectoring Potato leafroll virus (PLRV). *Neotropical Entomology*, 44: 604-609.
- Gasparic, H. V., Grubelic, M., Uzelac, V. D., Bazok, R., Cacija, M., Drmic, Z., & Lemic, D. 2020. Neonicotinoid residues in sugar beet plants and soil under different agro-climatic conditions. *Agriculture*, 1010: 484.
- Gilchrist, G. W., & Huey, R. B. 2001. Parental and developmental temperature effects on the thermal dependence of fitness in *Drosophila melanogaster*. *Evolution*, 55: 209-214.
- Giles, K. L., McCornack, B. P., Royer, T. A., & Elliott, N. C. 2017. Incorporating biological control into IPM decision making. *Current Opinion in Insect Science*, 20: 84-89.
- Gill, H., & McSorley, R. 2012. Methods for sampling soil surface arthropods in bush beans: Which one is the best? *Proceedings of the Florida State Horticultural Society*, 125: 192-195.
- Göllner-Scheiding, U. 1976. Revision der Gattung *Liorhyssus* Stål, 1870 (Heteroptera, Rhopalidae). *Deutsche Entomologische Zeitschrift*, 23: 181-206.
- Gómez, L., & Aguilar, E. 2016. *Guía de cultivo de la quinua*. Organización de las Naciones Unidas para la Alimentación y la Agricultura, Universidad Nacional Agraria La Molina.
- Gómez-Pando, L., Aguilar-Castellanos, E., & Ibañez-Tremolada, M. 2019. Quinoa (*Chenopodium quinoa* Willd.) breeding. In: Al-Khayri, J. M., Jain, S. M., & Johnson, D. V. (Eds.). Advances in plant breeding strategies: Cereals. Springer, pp. 259–316.
- Gómez-Pando, L., Mujica, A., Chura, Canahua, Pérez, Tejada, Villantoy, Pocco, Gonzáles, V., & Ccoñas, W. 2014. Perú: Capitulo Numero 5.2. In: Bazile, D., Bertero, D., Nieto, C. (Eds.). Estado del arte de la quinua en el mundo en 2013. FAO (Santiago de Chile) y CIRAD (Montpellier, Francia), pp. 450–461.

- González Olazo, E. V., & Reguilón, C. 2002. Una nueva especie de *Chrysoperla* (Neuroptera: Chrysopidae) para la Argentina. *Revista de La Sociedad Entomológica Argentina*, 61: 47–50.
- Gotelli, N., & Colwell, R. 2011. Estimating species richness. In: Magurran, A. E., & McGill, B. J. (Eds.). *Biological diversity: Frontiers in measurement and assessment*. Oxford University Press, pp. 39–54.
- Goulard, M. D., Specht, A., Sosa-Gomez, D. R., Roque-Specht, V. F., & De Barros, N. M. 2014. Immature stages of *Spodoptera eridania* (Lepidoptera: noctuidae): developmental parameters and host plants. *Journal of Insect Science*, 14: 1-11.
- Govindan, K., Gunasekaran, K., & Kuttalam, S. 2013. Emamectin Benzoate 5 SG: A safer insecticide to coccinellids predators in cotton ecosystem. *African Journal of Agricultural Research*, 8: 2455-2460.
- Gross, G. 1950. The stilt-bugs (Heteroptera-Neididae) of the Australian and New Zealand regions. *Records of the South Australian Museum*, 9: 313-326.
- Guerra García, H. (2006). *Agricultura peruana*, second edition. Asociación de Promoción Agraria, Lima, Peru.
- Guerrero, A. L., Gallucci, S. S., Michalijos, P., & Visciarelli, S. M. 2011. Países Andinos: Aportes teóricos para un abordaje integrado desde las perspectivas geográfica y turística. *Huellas*, 15: 121-138.
- Halloy, S., Ortega, R., Yager, K., & Seimon, A. 2005. Traditional Andean cultivation systems and implications for sustainable land use. *Acta Horticulturae*, 670: 31-55.
- Hansen, E. A., Funderburk, J. E., Reitz, S. R., Ramachandran, S., Eger, J. E., & McAuslane, H. 2003. Within-plant distribution of *Frankliniella* species (Thysanoptera: Thripidae) and *Orius insidiosus* (Heteroptera: Anthracoridae) in field pepper. *Environmental Entomology*, 32: 1035-1044.
- Haramboure, M., Mirande, L., & Schneider, M. I. 2015. Improvement of the mass rearing of larvae of the neotropical lacewing *Chrysoperla externa* through the incorporation of a new semiliquid artificial diet. *BioControl*, 61: 69-78.
- Harbhajan, K., & Kaur, S. 2017. DNA barcoding of Six Species of Family Rhopalidae (Insecta: Hemiptera: Heteroptera) from India. *International Journal of Life Sciences*, 5: 517-526.
- Hardy, M. C. 2011. Using selective insecticides in sustainable IPM. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 2, 6: 1-7.
- Hassan, S. A. 1985. Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms'. *Eppo Bulletin*, 15: 214–255.
- He, X., Wang, Q., & Carpenter, A. 2003. Thermal requirements for the development and reproduction of *Nysius huttoni* White (Heteroptera: Lygaeidae). *Journal of Economic Entomology*, 96: 1119-1125.
- He, Z., Guo, J. F., Reitz, S. R., Lei, Z. R., & Wu, S. Y. 2020. A global invasion by the thrip, *Frankliniella occidentalis*: Current virus vector status and its management. *Insect Science*, 27: 626-645.
- Heckman, C. 2017. *Neuroptera (Including Megaloptera)*. Springer, Washington, USA.

- Heie, O. E., Pettersson, J., Fuentes-Contreras, E., & Niemeyer, H. M. 1996. New records of aphids (Hemiptera: Aphidoidea) and their host-plants from northern Chile. *Revista Chilena de Entomología*, 23: 83-87.
- Heming, B. S. 1978. Structure and function of the mouthparts in larvae of *Haplothrips verbasci* (Osborn) (Thysanoptera, Tubulifera, Phlaeothripidae). *Journal of Morphology*, 156: 1-37.
- Hénault-Ethier, L. 2015. Health and environmental impacts of pyrethroid insecticides: What we know, what we don't know and what we should do about it. Executive Summary and Scientific Literature Review. Prepared for Équiterre. Montreal.
https://www.equiterre.org/sites/fichiers/health_and_environmental_impacts_of_pyrethroid_insecticides_full_report_en.pdf
- Henry, T., Dellapé, P., & de Paula, A. 2015. The big-eyed bugs, chinch bugs, and seed bugs (Lygaeoidea). In: Panizzi, A., & Grazia, J. (Eds.). True bugs (Heteroptera) of the Neotropics. Springer, Dordrecht, pp. 459–514.
- Hernández, L. M., & Henry, T. J. 2010. The plant bugs, or Miridae (Hemiptera: Heteroptera), of Cuba. Pensoft, Sofia, Bulgaria.
- Hervé, M. 2018. RVAideMemoire: Diverse basic statistical and graphical functions. R package version 0.9-70. <https://cran.r-project.org/web/packages/RVAideMemoire/RVAideMemoire.pdf>
- Hincapie, C. M., Saavedra, H. M., & Trochez, A. L. 1993. Life cycle, behaviour and natural enemies of *Liriomyza huidobrensis* (Blanchard) on bulb onion (*Allium cepa* L.). *Revista Colombiana de Entomología*, 19: 51-57.
- Hinojosa, L., Leguizamo, A., Carpio, C., Muñoz, D., Mestanza, C., Ochoa, J., Castillo, C., Murillo, A., Villacréz, E., Monar, C., Pichazaca, N., Murphy, K. 2021. Quinoa in Ecuador: Recent advances under global expansion. *Plants*, 10: 298.
- Hodkinson, I., & White, I. 1979. Handbooks for the Identification of British Insects: Homoptera: Psylloidea (Vol. 2). Royal Entomological Society of London: Queen's Gate, London, UK.
- Holland, J. M., Bianchi, F. J., Entling, M. H., Moonen, A. C., Smith, B. M., & Jeanneret, P. 2016. Structure, function and management of semi-natural habitats for conservation biological control: a review of European studies. *Pest management science*, 72: 1638-1651.
- Horn, D. 1983. Selective mortality of parasitoids and predators of *Myzus persicae* on collards treated with malathion, carbaryl, or *Bacillus thuringiensis*. *Entomologia Experimentalis et Applicata*, 34: 208-211.
- Horton, D., Miliczky, E., Lewis, T., Cooper, W., Waters, T., Wohleb, C., Zack, R., Johnson, D., & Jensen, A. 2018. New North American records for the old world psyllid *Heterotrioza chenopodii* (Reuter) (Hemiptera: Psylloidea: Triozidae) with biological observations. *Proceedings of the Entomological Society of Washington*, 120: 134-152.
- Hothorn, T., Bretz, F., Westfall, P., Heiberger, R., Schuetzenmeister, A., & Scheibe, S. 2022. Package 'multcomp': Simultaneous Inference in General Parametric Models, version 1.4-18.
<http://ftp5.gwdg.de/pub/misc/cran/web/packages/multcomp/multcomp.pdf>
- Hradil, K., Kment, P., & Roháčová, M. 2007. New records of *Liorhyssus hyalinus* (Heteroptera: Rhopalidae) in the Czech Republic, with a review of its worldwide distribution and biology. *Acta Musei Moraviae, Scientiae Biologicae*, 92: 53-107.

- Huang, N., & Enkegaard, A. 2010. Predation capacity and prey preference of *Chrysoperla carnea* on *Pieris brassicae*. *BioControl*, 55: 379-385.
- Hydorn, S. B., & Whitcomb, W. H. 1979. Effects of larval diet on *Chrysopa rufilabris*. *The Florida Entomologist*, 64: 293-298.
- IRAC. 2019. Pesticide resistance management. Insecticide Resistance Action Committee (IRAC). <https://www.irc-online.org/about/resistance/management/>
- Ishaaya, I., Barazani, A., Kontsedalov, S., & Horowitz, A. R. 2007. Insecticides with novel modes of action: Mechanism, selectivity and cross-resistance. *Entomological Research*, 37: 148-152.
- Ishaaya, I., & Degheele, D. 1998. Insecticides with novel modes of action: Mechanisms and application. Springer-Verlag, Heidelberg, Germany.
- Ishaaya, I., Kontsedalov, S., & Horowitz, A. 2002. Emamectin, a novel insecticide for controlling field crop pests. *Pest Management Science*, 58: 1091-1095.
- Ishaaya, I., Navon, A., & Gurevitz, E. 1986. Comparative toxicity of chlorfluazuron (IKI-7899) and cypermethrin to *Spodoptera littoralis*, *Lobesia botrana* and *Drosophila melanogaster*. *Crop Protection*, 5: 385-388.
- Jacobsen, S. E. 2011. The situation for quinoa and its production in southern Bolivia: From economic success to environmental disaster. *Journal of Agronomy and Crop Science*, 197: 390-399.
- Jacobsen, S. E., Mujica, A., & Jensen, C. R. 2003. The resistance of quinoa (*Chenopodium quinoa* Willd.) to adverse abiotic factors. *Food Reviews International*, 19: 99-109.
- Jafari, A. A., Fathipour, Y., Hosseini, S. M., Talebi, A. A., & Moharamipour, S. 2006. Preference of *Nabis capsiformis* and *Chrysoperla carnea* to different nymph instars of *Creontiades pallidus*. *Journal of Agricultural Sciences*, 12: 57-65.
- Jain, P., Singh, S. B., Borban, K., & Badaya, A. K. 2018. Bio-efficacy of novel insecticides against chilli aphid, *Aphis gossypii* Glover and thrips, *Scirtothrips dorsalis* Hood in Malwa region of Madhya Pradesh. *Annals of Plant and Soil Research*, 20: 172-177.
- Jansson, R. K., & Dybas, R. A. 1998. Avermectins: Biochemical mode of action, biological activity and agricultural importance. In: Ishaaya, I., & Degheele, D. (Eds.). Insecticides with novel modes of action: Mechanisms and application. Springer, Berlin, Germany, pp. 152–170.
- Jensen, S. 2000. Insecticide resistance in the western flower thrips, *Frankliniella occidentalis*. *Integrated Pest Management Reviews*, 5: 131-146.
- Jeschke, P., Witschel, M., Krämer, W., & Schirmer, U. 2019. Modern crop protection compounds Volume 3: Insecticides. Wiley-VCH, Weinheim, Germany.
- Joas, R., & Cotillon, A. 2009. Development of guidance for establishing Integrated Pest Management (IPM) principles. Final report. European Commission.
- Kar, A. 2017. Bioefficacy evaluation of imidacloprid 17.8% SL and thiamethoxam against whitefly on tomato and their effect on natural enemies. *Journal of Entomology and Zoology Studies*, 5: 1064-1067.

- Karsholt, O. 1994. Some moths introduced into Denmark, with remarks on this subject (Lepidoptera). *Entomologiske Meddelelser*, 62: 1-6.
- Kavallieratos, N., Tomanović, N., Starý, P., Žikić, V., & Petrović-Obradović, O. 2010. Parasitoids (Hymenoptera: Braconidae: Aphidiinae) attacking aphids feeding on Solanaceae and Cucurbitaceae crops in southeastern Europe: Aphidiine-aphid-plant associations and key. *Annals of the Entomological Society of America*, 103: 153-164.
- Kavallieratos, N., Tomanović, Ž., Petrović, A., Janković, M., Starý, P., Yovkova, M., & Athanassiou, C. 2013. Review and key for the identification of parasitoids (Hymenoptera: Braconidae: Aphidiinae) of aphids infesting herbaceous and shrubby ornamental plants in southeastern Europe. *Annals of the Entomological Society of America*, 106: 294-309.
- Kehat, M., & Wyndham, M. 1972. The influence of temperature on development, longevity, and fecundity in the Rutherglen bug, *Nysius vinitor* (Hemiptera: Lygaeidae). *Australian Journal of Zoology*, 20: 67-78.
- Kerzhner, I. M., & Henry, T. J. 2008. Three new species, notes and new records of poorly known species, and an updated checklist for the North American Nabidae (Hemiptera: Heteroptera). *Proceedings of the Entomological Society of Washington*, 110: 988-1011.
- Khani, A., Ahmadi, F., & Ghadamyari, M. 2012. Side effects of imidacloprid and abamectin on the mealybug destroyer *Cryptolaemus montrouzieri*. *Trakia Journal of Sciences*, 10: 30-35.
- Kindt, R. 2018. Biodiversity R: package for community ecology and suitability analysis. R version, 2.10-1. <https://cran.r-project.org/web/packages/BiodiversityR/index.html>
- King, A. B., & Saunders, J. L. 1984. The invertebrate pests of annual food crops in Central America. A guide to their recognition and control. Overseas Development Administration, London, UK.
- Kontodimas, D. C., Eliopoulos, P. A., Stathas, G. J., & Economou, L. P. 2004. Comparative temperature-dependent development of *Nephus includens* (Kirsch) and *Nephus bisignatus* (Boheman) (Coleoptera: Coccinellidae) preying on *Planococcus citri* (Risso) (Homoptera: Pseudococcidae): evaluation of a linear and various nonlinear models using specific criteria. *Environmental Entomology*, 33: 1-11.
- Korytkowski, C. 2014. Contribución al conocimiento de los Agromyzidae (Diptera: Muscomorpha) en el Perú. *Revista Peruana de Entomología*, 49: 1-106.
- Krysan, J., Branson, T., Schroeder, R., & Steiner Jr., W. 1984. Elevation of *Diabrotica sicuanica* (Coleoptera: Chrysomelidae) to the species level with notes on the altitudinal distribution of *Diabrotica* species in the Cuzco department of Peru. *Entomological News*, 95: 91-98.
- Labronici - Bertin, R. L., Gonzaga, L. V., Campelo, G., Azevedo, M. S., Maltez, H. F., Heller, M., Micked, G., Ballod, L. B., & Fett, R. 2014. Nutrient composition and, identification/quantification of major phenolic compounds in *Sarcocornia ambigua* (Amaranthaceae) using HPLC–ESI-MS/MS. *Food Research International*, 55: 404-411.
- Lafontaine, J. D., & Schmidt, B. C. 2010. Annotated check list of the Noctuoidea (Insecta, Lepidoptera) of North America north of Mexico. *ZooKeys*, 40: 1-239.

- Lambrot, L., Guerrero, M. A., & Araya, J. E. 1999. Lepidópteros asociados al cultivo de la quinoa (*Chenopodium quinoa* Willd.) en la zona central de Chile. *Boletín de Sanidad Vegetal Plagas*, 25: 203-207.
- Lanzoni, A., Bazzocchi, G. G., Burgio, G., & Fiacconi, M. R. 2002. Comparative life history of *Liriomyza trifolii* and *Liriomyza huidobrensis* (Diptera: Agromyzidae) on beans: Effect of temperature on development. *Environmental Entomology*, 31: 797–803.
- Latorre, J. 2017. Is quinoa cultivation on the coastal desert of Peru sustainable? A case study from Majes, Arequipa [Master thesis], Aarhus University, Denmark.
- Leather, S. 2005. Insect sampling in forest ecosystems. Blackwell Science Ltd, United Kingdom.
- Letourneau, D. K., & Goldstein, B. 2001. Pest damage and arthropod community structure in organic vs. Conventional tomato production in California. *Journal of Applied Ecology*, 38: 557–570.
- Livia, C., & Sánchez, G. 2019. Soil insects associated with lucumo (*Pouteria lucuma* L.) trees in La Molina, Lima, Peru. *Peruvian Journal of Agronomy*, 3: 16-23.
- Livia, C., & Sánchez, G. 2020. Soil arthropods associated with sweetpotato crop (*Ipomoea batata* L.) in La Molina, Lima, Peru. *Peruvian Journal of Agronomy*, 4: 1-9.
- Livia, C., Sánchez, G., & Cruces, L. 2020. Diversidad de insectos del suelo asociados al cultivo de maíz (*Zea mays* L.) en La Molina/Lima/Perú. *Ecología Aplicada*, 19: 57-64.
- López, R., Carmona, D., Vincini, A., Monterubbianesi, G., & Caldiz, D. 2010. Population dynamics and damage caused by the leafminer *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae), on seven potato processing varieties grown in temperate environment. *Neotropical Entomology*, 39: 108-114.
- López-Arroyo, J. I., Tauber, C. A., & Tauber, M. J. 1999. Effects of prey on survival, development, and reproduction of trash-carrying chrysopids (Neuroptera: Ceraeochrysa). *Environmental Entomology*, 28: 1183-1188.
- Luna-Espino, H. M., Jiménez-Pérez, A., & Castrejón-Gómez, V. R. 2020. Assessment of *Chrysoperla comanche* (Banks) and *Chrysoperla externa* (Hagen) as biological control agents of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) on tomato (*Solanum lycopersicum*) under glasshouse conditions. *Insects*, 11: 87.
- Luypaert, G., Witters, J., Van Huylbroeck, J., Maes, M., De Riek, J., & De Clercq, P. 2014. Temperature-dependent development of the broad mite *Polyphagotarsonemus latus* (Acari: Tarsonemidae) on *Rhododendron simsii*. *Experimental and Applied Acarology*, 63: 389-400.
- Magurran, A. 2004. *Measuring biological diversity*. Blackwell Science Ltd, United Kingdom.
- Mahmood, I., Imadi, S. R., Shazadi, K., Gul, A., & Hakeem, K. R. 2016. Effects of pesticides on environment. In: Hakeem, K. R., & Akhtar, M. S. (Eds.). *Plant, soil and microbes*. Springer, Cham, pp. 253–269.
- Malavolta, C., Boller, E. F., & Wijnands, F. G. 2005. Guidelines for integrated production of field grown vegetables. *Bulletin OIBC/WPRS*, 28: 1-24.

- Mamani, A. E. 2015. Determinación del efecto de tres dietas en el ciclo y parámetros biológicos del *Nysius* sp. (Hemiptera. Lygaeidae) chinche de semilla en el laboratorio [Bachelor thesis]. Universidad Nacional de San Agustín, Arequipa, Peru.
- Mamani, D. 1998. Control biológico en forma natural de la polilla de la quinua (*Eurysacca melanocampta* Meyrick) por parasitoides y perspectivas de cría para su manipulación en el Altiplano Central [Bachelor thesis]. Universidad Mayor de San Andrés, La Paz, Bolivia.
- Manjula, K. N., & Kotikal, Y. K. 2018. Evaluation of insecticides against *Agrotis segetum* (Denis and Schiffermuller) and *Spoladea recurvalis* (Fabricius) on fenugreek, *Trigoniella foenumgraecum* L. *Journal of Entomology and Zoology Studies*, 6: 1177-1182.
- Manly, B. F. J. 1974. A model for certain types of selection experiments. *Biometrics*, 30: 281–294.
- Maquera, W. A. 2018. Ciclo biológico de *Nysius simulans* (Stål, 1860) (Hemiptera: Lygaeidae) en variedades comerciales de quinua en condiciones de laboratorio [Master thesis]. Universidad Nacional Agraria La Molina, Lima, Peru.
- Marca, W. 2015. Comparativo de rendimiento de 12 variedades de quinua (*Chenopodium quinoa* Willd.) en siembra de verano, en el Centro Experimental Agrícola III Los Pichones–Tacna [Bachelor thesis]. Universidad Nacional Jorge Basadre Grohmann, Tacna, Peru.
- Maredia, K. M., Dakouo, D., & Mota-Sanchez, D. 2003. Integrated pest management in the global arena. CABI, Wallingford.
- Margalef, R. 1972. Homage to Evelyn Hutchinson, or why there is an upper limit to diversity. *Connecticut Academy of Arts and Sciences*, 44: 214–235.
- Marschner, I., & Donoghoe, M. 2018. Package 'glm2': Fitting Generalized Linear Models, Version 1.2.1. <http://mirror.psu.ac.th/pub/cran/web/packages/glm2/glm2.pdf>
- Martínez, L. C., Plata-Rueda, A., Gonçalves, W. G., Freire, A. F., Zanuncio, J. C., Bozdoğan, H., & Serrão, J. E. 2019. Toxicity and cytotoxicity of the insecticide imidacloprid in the midgut of the predatory bug, *Podisus nigrispinus*. *Ecotoxicology and Environmental Safety*, 167: 69-75.
- Masner, L. 1976. Revisionary notes and keys to world genera of Scelionidae (Hymenoptera: Proctotrupoidea). *The Memoirs of the Entomological Society of Canada*, 108: 1-87.
- Maughan, P. J., Bonifacio, A., Coleman, C. E., Jellen, E. N., Stevens, M. R., & Fairbanks, D. J. 2007. Quinoa (*Chenopodium quinoa*). In: Kole, C. (Ed.). Pulses, sugar and tuber crops. Springer, Berlin, Germany, pp. 147–158.
- McDonell, E. 2019. (Re) producing "Indian Food": Race, value, and development in Peru's quinoa boom-bust [Doctoral dissertation]. Indiana University, Bloomington, Indiana.
- McDougall, S., Watson, A., Stodart, B., Napier, T., Kelly, G., Troidahl, D., & Tesoriero, L. 2013. Tomato, capsicum, chilli and eggplant. A field guide for the identification of insect pests, beneficials, diseases and disorders in Australia and Cambodia. The Australian Centre for International Agricultural Research (ACIAR), Canberra.
- McEwen, P., New, T., & Whittington, A. E. 2007. Lacewings in the crop environment. Cambridge University Press, New York.

- McLaughlin, A., & Mineau, P. 1995. The impact of agricultural practices on biodiversity. *Agriculture, Ecosystems & Environment*, 55: 201-212.
- Mead, H. M., & Khedr, M. M. 2018. Role of teflubenzuron as a chitin synthesis inhibitor against *Spodoptera littoralis* larvae. *Egyptian Academic Journal of Biological Sciences*, 10: 49-58.
- Menalled, F. D., & Landis, D. A. 2008. Conservation of ground beetles in annual crops. In: Capinera, J. (Ed.). *Encyclopedia of entomology*, second edition. Springer Science & Business Media, Gainesville, pp. 1023–1025.
- Mercado, W., & Ubillus, K. 2017. Characterization of producers and quinoa supply chains in the Peruvian regions of Puno and Junín. *Scientia Agropecuaria*, 8: 251-265.
- Miller, S. O. 2019. Management of *Spoladea recurvalis* (Lepidoptera: Crambidae) on amaranths using biopesticides [Doctoral dissertation]. North-West University, Potchefstroom, Sudáfrica.
- Mills, N. 2006. Interspecific competition among natural enemies and single versus multiple introductions in biological control. In: Brodeur, J., & Boivin, G. (Eds.). *Trophic and guild in biological interactions control*. Springer, Dordrecht, pp. 191–220.
- Mohammad, N. 2014. An overview of the IPM techniques for saving the agricultural biodiversity in Malaysia. *Journal of Applied Science and Agriculture*, 9: 2666-2671.
- Mohammed, A. A., Desneux, N., Fan, Y., Han, P., Ali, A., Song, D., & Gao, X. 2018. Impact of imidacloprid and natural enemies on cereal aphids: Integration or ecosystem service disruption? *Entomologia Generalis*, 37: 47-61.
- Molina-Ochoa, J.; Hutchison, W. D., & Blanco, C. A. 2010. Current status of *Helicoverpa zea* and *Heliothis virescens* within a changing landscape in the southern United States and Mexico. *Southwestern Entomologist*, 5: 347-354.
- Molinari, A. M., & Gamundi, J. C. 2010. La “chinche diminuta” *Nysius simulans* en soja. *INTA EEA Oliveros*, 45: 117–120.
- Monteiro, R. C., Mound, L. A., & Zucchi, R. A. 1999. Thrips (Thysanoptera) as pests of plant production in Brazil. *Revista Brasileira de Entomologia*, 43: 163-171.
- Montero, C., & Armando, C. 2017. Análisis económico de la producción nacional de quinua. Ministerio de Agricultura y Riego, Lima, Peru. <https://www.minagri.gob.pe/portal/analisis-economico/analisis-2017?download=12316:boletin-de-quinua>
- Montero, G.; Vignaroli, L.; Cavaglia, S., & Lietti, M. 2007. Colza, algo nuevo en la región. *Revista Agromensajes de la Facultad de Ciencias Agrarias*, Universidad Nacional de Rosario. Argentina. <http://rephip.unr.edu.ar/bitstream/handle/2133/897/Colza%2C%20algo%20nuevo%20en%20la%20regi%C3%B3n.pdf?sequence=1>
- Montezano, D. G., Specht, A., Sosa-Gómez, D. R., Roque-Specht, V. F., & de Barros, N. M. 2013. Biotic potential and reproductive parameters of *Spodoptera eridania* (Stoll) (Lepidoptera: Noctuidae) in the laboratory. *Revista Brasileira de Entomologia*, 57: 340–346.
- Montoro, Y., Moreno, R., Gomero, L., & Reyes, M. 2009. Características de uso de plaguicidas químicos y riesgos para la salud en agricultores de la sierra central del Perú. *Revista Peruana de Medicina Experimental y Salud Pública*, 26: 466-472.

- Montoya, J. M., Pimm, S. L., & Solé, R. 2006. Ecological networks and their fragility. *Nature*, 442: 259-264.
- Moreno, C., & Halffter, G. 2000. Assessing the completeness of bat biodiversity inventories using species accumulation curves. *Journal of Applied Ecology*, 37: 149-158.
- Moret, P. 1995. Contribution à la connaissance du genre néotropical *Blennidus* Motschulsky, 1865. *Bulletin de La Société Entomologique de France*, 100: 489-500.
- Moret, P. 2003. Clave de identificación para los géneros de Carabidae (Coleoptera) presentes en los páramos del Ecuador y del sur de Colombia. *Revista Colombiana de Entomología*, 29: 185-190.
- Motaung, T. E. 2020. Chloronicotinyl insecticide imidacloprid: Agricultural relevance, pitfalls and emerging opportunities. *Crop Protection*, 131: 105097.
- Motswagole, R., Gotcha, N., & Nyamukondiwa, C. 2019. Thermal biology and seasonal population abundance of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae): Implications on pest management. *International Journal of Insect Science*, 11: 1-9.
- Mouhoubi, D., Djenidi, R., & Bounechada, M. 2019. Contribution to the study of diversity, distribution, and abundance of insect fauna in salt wetlands of Setif Region, Algeria. *International Journal of Zoology*, 2019: 212841.
- Mound, L. A., & Kibby, G. 1998. Thysanoptera: An identification guide, second edition. Cab International, Wallingford, USA.
- Mound, L. A., & Marullo, R. 1996. The Thrips of Central and South America: An introduction (Insecta: Thysanoptera). *Memoirs on Entomology, International*, Volume 6.
- Mound, L. A., & Ng, Y. F. 2009. An illustrated key to the genera of Thripinae (Thysanoptera) from South East Asia. *Zootaxa*, 2265: 27-47.
- Mousseau, T. A., & Dingle, H. 1991. Maternal effects in insect life histories. *Annual Review of Entomology*, 36: 511-534.
- Mroczek, A. 2015. Phytochemistry and bioactivity of triterpene saponins from Amaranthaceae family. *Phytochemistry Reviews*, 14: 577-605.
- Mujica, A. 1994. Andean grains and legumes. In: Bermejo, J. E. H., & León, J. (Eds.). *Neglected crops: 1492 from a different perspective*. FAO Plant Production and Protection Series, Rome, Italy, pp. 131-148.
- Mujica, A., & Canahua, A. 1989. Fases fenológicas del cultivo de la quinua (*Chenopodium quinoa* Willd.). In: Mujica, A., & Canahua, A. (Eds.). *Fenología de cultivos andinos y uso de la información agrometeorológica*. INIAA, EEZA-ILLPA, PICA, PISA, Puno, Peru, pp. 23-27.
- Mujica, N., & Kroschel, J. 2011. Leafminer fly (Diptera: Agromyzidae) occurrence, distribution, and parasitoid associations in field and vegetable crops along the Peruvian coast. *Environmental Entomology*, 40: 217-230.
- Mujica, N., Sporleder, M., Carhuapoma, P., & Kroschel, J. 2017. A temperature-dependent phenology model for *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Journal of Economic Entomology*, 110: 1333-1344.

- Mujica, N., Valencia, C., Ramirez, L., Prudencio, C., & Kroschel, J. 2009. Temperature-dependent development of three parasitoids of the leafminer fly *Liriomyza huidobrensis* [Conference]. Tropical roots and tubers in a changing climate: a convenient opportunity for the world. Fifteenth Triennial Symposium of the International Society for Tropical Root Crops, Lima, Peru. http://www.istrc.org/images/Documents/Symposiums/Fifteenth/s7_mujica.pdf
- Mullan, B., Tait, A., & Thompson, C. 2006. 'Climate—New Zealand's climate', Te Ara—The Encyclopedia of New Zealand. <http://www.TeAra.govt.nz/en/climate/page-1>
- Muralikrishna, P., Mathew, T. B., Paul, A., & Nithya, P. R. 2019. Evaluation of bio-efficacy of new generation insecticides, botanicals and microbial insecticides on leaf webber of amaranth. *Journal of Entomology and Zoology Studies*, 7: 516-520.
- Muneret, L., Mitchell, M., Seufert, V., Aviron, S., Djoudi, E. A., Pétilion, J., Plantegenest, M., Thiéry, D., & Rusch, A. 2018. Evidence that organic farming promotes pest control. *Nature Sustainability*, 1: 361-368.
- Muthaiyan, M. C. 2009. Principles and practices of plant quarantine. Allied Publishers Private Limited, New Delhi.
- Nakahara, S. 1993. Syllabus for Thysanoptera larvae. The 1993 International Conference on Thysanoptera: Thrips Identification Workshop, Burlington, USA.
- Nakamura, S., Masuda, T., Mochizuki, A., Konishi, K., Tokumaru, S., Ueno, K., & Yamaguchi, T. 2013. Primer design for identifying economically important *Liriomyza* species (Diptera: Agromyzidae) by multiplex PCR. *Molecular Ecology Resources*, 13: 96-102.
- National Research Council. 1989. Lost crops of the Incas: Little-known plants of the Andes with promise for worldwide cultivation. National Academies Press, Washington, USA.
- Naumann, K. 2012. Synthetic pyrethroid insecticides: Chemistry and patents. Springer, Heidelberg.
- Navarrete-Heredia, J., Newton, A., Thayer, M., Ashe, J., & Chandler, D. 2002. Guía ilustrada de los Staphylinidae (Coleoptera) de México. Illustrated Guide to the Genera of Staphylinidae (Coleoptera) of Mexico. Universidad de Guadalajara-CONABIO, Guadalajara, Mexico.
- Navon, A. 2000. *Bacillus thuringiensis* application in agriculture. In: Charles, F., Delécluse, A., & Nielsen-LeRoux, C. (Eds.). Entomopathogenic bacteria: From laboratory to field application. Springer, Dordrecht, pp. 355–369.
- Neuen, R. 1995. Behaviour modifying effects of low systemic concentrations of imidacloprid on *Myzus persicae* with special reference to an antifeeding response. *Pesticide Science*, 44: 145-153.
- Nguyen, V. H., Jonckheere, W., Nguyen, D. T., de Moraes, G. J., Van Leeuwen, T., & De Clercq, P. 2019. Phytoseiid mites prey effectively on thrips eggs: Evidence from predation trials and molecular analyses. *Biological Control*, 137: 104012.
- Nordlund, D. A., & Morrison, R. K. (1990). Handling time, prey preference, and functional response for *Chrysoperla rufilabris* in the laboratory. *Entomologia Experimentalis et Applicata*, 57: 237-242.

- Núñez, E. 2016. Estudio de la diversidad fenotípica del maíz (*Zea mays* L.) en la sierra baja y media del Perú [Bachelor thesis]. Universidad Nacional Agraria La Molina, Lima, Peru.
- Ochoa-Vizarreta, R., & Franco-Navia, J. 2013. Morfología y biología de la polilla de la quinua *Eurysacca melanocampta* Meyrick, 1917, (Lepidoptera: Gelechiidae), de Cusco. *Bioma*, 1: 35-38.
- OEEE, (Oficina de Estudios Económicos y Estadística). 2012. Producción agrícola 2012. Ministerio de agricultura y riego del Perú. <http://siea.minagri.gob.pe/siea/?q=publicaciones/anuario-de-produccion-pecuaria>
- Oksanen, J. 2009. Multivariate analysis of ecological communities in R: vegan tutorial. http://brianmcgill.org/614/reading/vegan_tutorial.pdf
- Oksanen, J., Blanchet, F., Michael, F., Kindt, R., Legendre, P., McGlinn, D., Minchin, R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H., Szoecs, E., Wagner, H. 2020. Package 'vegan'. Community ecology package, version 2.5(6). <https://cran.r-project.org/web/packages/vegan/vegan.pdf>
- Oliver, I., & Beattie, A. J. 1993. A possible method for the rapid assessment of biodiversity. *Conservation Biology*, 7: 562–568.
- Oliver, I., & Beattie, A. J. 1996. Invertebrate morphospecies as surrogates for species: A case study. *Conservation Biology*, 10: 99–109.
- Orellano, H., & Tillmann, H. 1984. La quinua en Yanamarca, prov. de Jauja: Testimonios sobre la siembra campesina. *Boletín de Lima*, 6: 55-64.
- Osman, M. Z., & Selman, B. J. 1996. Effect of larval diet on the performance of the predator *Chrysoperla carnea* Stephens (Neuropt., Chrysopidae). *Journal of Applied Entomology*, 120: 115-117.
- Pacheco-Rueda, I., Lomeli-Flores, J. R., López-Arroyo, J. I., González-Hernández, H., Romero-Napoles, J., Santillán-Galicia, M., Suárez-Espinoza, J. 2015. Preferencia de tamaño de presa en seis especies de Chrysopidae (Neuroptera) sobre *Diaphorina citri* (Hemiptera: Liviidae). *Revista Colombiana de Entomología*, 41: 187-193.
- Pall, J., Kihn, R., & Diez, F. 2016. A review of genus *Nysius* Dallas in Argentina (Hemiptera: Heteroptera: Orsillidae). *Zootaxa*, 4132: 221-234.
- Pande, Y. D. 1969. Biology of *Hymenia recurvalis* Fabricius (Pyralidae: Lepidoptera) as a defoliator of the serious «kharif» weeds in Rajasthan. *Indian Journal of Science and Industry*, 3: 107-108.
- Pande, Y. D. 1972. Some observations on the bionomics of *Hymenia recurvalis* F. (Lepid., Pyralidae) feeding on *Trianthema monogyna* and *Amaranthus viridis* in India. *Zeitschrift Für Angewandte Entomologie*, 72: 362-366.
- Panizzi, A., & Grazia, J. 2015. True bugs (Heteroptera) of the Neotropics. Springer, Dordrecht.
- Pappas, M. L., Broufas, G. D., & Koveos, D. S. 2007. Effects of various prey species on development, survival and reproduction of the predatory lacewing *Dichochrysa prasina* (Neuroptera: Chrysopidae). *Biological Control*, 43: 163-170.
- Parker, B. L., Skinner, M., & Lewis, T. 1995. Thrips biology and management. Springer Science & Business Media, New York.

- Parrella, M. P., Allen, W. W., & Morishita, P. 1981. Leafminer species causes California mum growers new problems. *California Agriculture*, 35: 28-30.
- Pastrana, J. A. 2004. Los lepidópteros argentinos: Sus plantas hospedadoras y otros substratos alimenticios. Sociedad Entomológica Argentina, Buenos Aires, Argentina.
- Peacock, L., & Worner, S. 2008. Biological and ecological traits that assist establishment of alien invasive insects. *New Zealand Plant Protection*, 61: 1-7.
- Perez-Alvarez, R., Nault, B. A., & Poveda, K. 2019. Effectiveness of augmentative biological control depends on landscape context. *Scientific Reports*, 9: 1-15.
- Peshin, R., & Zhang, W. 2014. Integrated pest management and pesticide use. In: Pimentel, D., & Peshin, R. (Eds.). *Integrated pest management*. Springer, Dordrecht, pp. 1–46.
- Pitterna, T. 2019. Glutamate-gated chloride channel allosteric modulators: Avermectins and milbemycins. In: Jeschke, P., Witschel, M., Krämer, W., & Schirmer, U. (Eds.). *Modern crop protection compounds*. Wiley-VCH, Weinheim, Germany, pp. 1478–1501.
- Pogue, M. G. 2002. A World Revision of the Genus *Spodoptera* Guenée (Lepidoptera: Noctuidae). *Memoirs of the American Entomological Society*, 43: 1-202.
- Pogue, M. G. 2013. Revised status of *Chloridea* Duncan and (Westwood), 1841, for the *Heliothis virescens* species group (Lepidoptera: Noctuidae: Heliiothinae) based on morphology and three genes. *Systematic Entomology*, 38: 523–542.
- Pons, X., & Albajes, R. 2001. Density of epigeal predators on maize plants untreated and treated with imidacloprid. *IOBC WPRS Bulletin*, 24: 73-78.
- Passoa, S. C. 2014. Identification guide to larval Heliiothinae (Lepidoptera: Noctuidae) of quarantine significance. USDA/APHIS/PPQ, Columbus, OHIO.
http://idtools.org/id/leps/lepintercept/LepIntercept_Heliiothinae.pdf
- Povolný, D. 1979. On some little-known moths of the family Gelechiidae (Lepidoptera) as pests of crops. *Acta Universitatis Agriculturae, Facultad Agronómica*, 27:139-165.
- Povolný, D. 1986. Gnorimoschemini of Southern South América. II the Genus *Eurysacca* (Lepidoptera gelechiidae). *Steenstrupia*, 12: 1-47.
- Povolný, D. 1990. Gnorimoschemini of Perú and Bolivia (Lepidoptera, Gelechiidae). *Steenstrupia*, 16: 153-223.
- Povolný, D. 1997. *Eurysacca quinoa* sp. A new quinoa-feeding species of the tribe Gnorimoschemini (Lepidoptera, Gelechiidae) from Bolivia. *Steenstrupia*, 22: 41-43.
- Povolný, D., & Valencia, L. 1986. Una palomilla de papa nueva para Colombia. In: Valencia, L. (Ed.). *Memorias del Curso sobre Control Integrado de Plagas de Papa*, Bogotá, Colombia, pp. 33–35.
- Powell, J. A., & Opler, P. A. 2009. *Moths of Western North America*. University of California Press, Berkeley.
- Prabhaker, N., Castle, S. J., Naranjo, S. E., Toscano, N. C., & Morse, J. G. 2011. Compatibility of two systemic neonicotinoids, imidacloprid and thiamethoxam, with various natural enemies of agricultural pests. *Journal of Economic Entomology*, 104: 773-781.

- Prado, E. 2008. Conocimiento actual de Hemiptera-Heteroptera de Chile con lista de especies. *Boletín Del Museo Nacional de Historia Natural, Chile*, 57: 31-75.
- Price, P. W., Denno, R. F., Eubanks, M. D., Finke, D. L., & Kaplan, I. 2011. *Insect ecology: Behavior, populations and communities*. Cambridge University Press, Cambridge.
- Pulgar Vidal, J. 1981. *Geografía del Perú: Las ocho regiones naturales del Perú*. Editorial Universo, Lima, Peru.
- Quirós, D. I., Remaudière, G., & Nieto-Nafría, J. M. 2009. Contribution to the knowledge of the Aphididae and Phylloxeridae (Hemiptera: Sternorrhyncha) from Panama. *Neotropical Entomology*, 38: 791-800.
- Quispe, R., Saravia, R., Villca, M., & Lino, V. 2014. Complejo Polilla. In: Saravia, R., Plata, G., & Gandarillas, A. (Eds.). *Plagas y enfermedades del cultivo de quinua*. Fundación PROINPA, Cochabamba, Bolivia, pp. 49–62.
- R Core Team. 2020. *R: A Language and Environment for Statistical Computing* [R Foundation for Statistical Computing], Vienna, Austria.
- Ramani, S. 2013. *Insect biodiversity and conservation of natural enemies in integrated pest management*. Central Potato Research Station, Meghalaya, http://kiran.nic.in/pdf/publications/pest_management.pdf
- Ramirez-Hernandez, A., Galagarza, O. A., Álvarez Rodriguez, M. V., Pachari Vera, E., Valdez Ortiz, M., Deering, A. J., Oliver, H. F. 2020. Food safety in Peru: A review of fresh produce production and challenges in the public health system. *Comprehensive Reviews in Food Science and Food Safety*, 19: 3323-3342.
- Rapisarda, C., & Cocuzza, G. E. M. 2017. *Integrated pest management in tropical regions*. CABI, Wallingford, UK.
- Rasmussen, C., Jacobsen, S. E., & Lagnaoui, A. 2001. Las polillas de la quinua (*Chenopodium quinoa* Willd.) en el Perú: *Eurysacca* (Lepidoptera: Gelechiidae). *Revista Peruana de Entomología*, 42: 57-59.
- Rasmussen, C., Lagnaoui, A., & Esbjerg, P. 2003. Advances in the knowledge of quinoa pests. *Food Reviews International*, 19: 61–75.
- Radio, P. A. 1928. Studies on the biology of the genus *Corizus* (Coreidae, Hemiptera). *Annals of the Entomological Society of America*, 21: 189-201.
- Reddy, P. P. 2014. *Biointensive integrated pest management in horticultural ecosystems*. Springer Science & Business Media, New Delhi.
- Reina, P., & La Salle, J. 2003. Key to the world genera of Eulophidae parasitoids (Hymenoptera) of leafmining Agromyzidae (Diptera). World Wide Web Electronic Publication. http://www.ento.csiro.au/science/eulophid_key/eulophids.htm.
- Reitz, S. R. 2009. Biology and ecology of the western flower thrips (Thysanoptera: Thripidae): The making of a pest. *Florida Entomologist*, 92: 7-13.
- Repo-Carrasco, R., Espinoza, C., & Jacobsen, S. E. 2003. Nutritional value and use of the Andean crops quinoa (*Chenopodium quinoa*) and kaniwa (*Chenopodium pallidicaule*). *Food Reviews International*, 19: 179–189.

- Ricupero, M., Desneux, N., Zappalà, L., & Biondi, A. 2020. Target and non-target impact of systemic insecticides on a polyphagous aphid pest and its parasitoid. *Chemosphere*, 247: 125728.
- Riddick, E. W. 2008. Ground beetle (Coleoptera: Carabidae) feeding ecology. In: Capinera, J. (Ed.). *Encyclopedia of entomology*, second edition. Springer Science Business Media, Gainesville, pp. 1742–1747.
- Ridgway, R. L., & Jones, S. L. 1968. Field-cage releases of *Chrysopa carnea* for suppression of populations of the bollworm and the tobacco budworm on cotton. *Journal of Economic Entomology*, 61: 892-898.
- Rinehart, T. A., & Boyd, D. W. 2006. Rapid, high-throughput detection of azalea lace bug (Hemiptera: Tingidae) predation by *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), using fluorescent-polymerase chain reaction primers. *Journal of Economic Entomology*, 99: 2136-2141.
- Ríos, R. 2014. Determinación, ciclo biológico, parámetros biológicos y comportamiento de *Liorhyssus hyalinus* (Fabricus, 1974) (Hemiptera: Rhopalidae) “chinche grande de la quinua”, Arequipa [Bachelor thesis]. Universidad Nacional de San Agustín, Arequipa, Peru.
- Ripley, B., Venables, B., Bates, D., Hornik, K., Gebhardt, A., Firth, D., & Ripley, M. 2020. Package ‘mass’. CRAN Repos. *Httpcran R-Proj*. <https://cran.r-project.org/web/packages/MASS/MASS.pdf>
- Rojas, W., & Patiño, F. 2014. Zonas agroecológicas de producción de quinua. In: Saravia, R., Plata, G., & Gandarillas, A. (Eds.). *Plagas y enfermedades de la quinua*. Fundación PROINPA, Cochabamba, Bolivia, pp. 13-16.
- Roubos, C. R., Rodriguez-Saona, C., Holdcraft, R., Mason, K. S., & Isaacs, R. 2014. Relative toxicity and residual activity of insecticides used in blueberry pest management: Mortality of natural enemies. *Journal of Economic Entomology*, 107: 277-285.
- Sabahi, Q., Rasekh, A., & Michaud, J. 2011. Toxicity of three insecticides to *Lysiphlebus fabarum*, a parasitoid of the black bean aphid, *Aphis fabae*. *Journal of Insect Science*, 11: 104.
- Salas, J., Alvarez, C., Parra, A., & Mendoza, O. 1988. Biología y hábitos de vida de *Liriomyza huidobrensis* Blanchard el pasador de la hoja de la papa (*Solanum tuberosum*). *Agronomía Tropical*, 38: 57-68.
- Samways, M. 2005. *Insect diversity conservation*. Cambridge University Press, Cambridge.
- Sánchez, G. 2006. *Manejo integrado de plagas en el Perú*. Universidad Nacional Agraria La Molina, Lima, Peru.
- Sánchez, G., & Redolfi de Huiza, I. 1988. *Liriomyza huidobrensis* y sus parasitoides en papa cultivada en Rímac y Cañete, 1986. *Revista Peruana de Entomología*, 31: 110-112.
- Sánchez, G., & Sánchez, J. 2008. *Manejo integrado del cultivo del espárrago en el Perú*. Instituto Peruano del Espárrago y Hortalizas, Lima, Peru.
- Sánchez, G. & Sarmiento, J. 2002. *Plagas del cultivo de algodónero*. Universidad Nacional Agraria La Molina, Lima, Peru.
- Sánchez, G., & Vergara, C. 2002. *Plagas de los cultivos andinos*, second edition. Universidad Nacional Agraria La Molina, Lima, Peru.

- Sánchez, G., & Vergara, C. 2003. Plagas de hortalizas. Universidad Nacional Agraria La Molina, Lima, Peru.
- Sánchez, G., & Vergara, C. 2005. Control biológico aplicado, second edition. Universidad Nacional Agraria La Molina, Lima, Peru.
- Sánchez-Bayo, F., Tennekes, H. A., & Goka, K. 2013. Impact of systemic insecticides on organisms and ecosystems. In: Trdan, S. (Ed.). Insecticides-development of safer and more effective technologies. InTech, Rijeka, Croatia, pp. 365–414.
- Saravia, R., Plata, G., & Gandarillas, A. 2014. Plagas y enfermedades del cultivo de quinua. Fundación PROINPA, Cochabamba, Bolivia.
- Saska, P., van der Werf, W., Hemerik, L., Luff, M. L., Hatten, T. D., & Honek, A. 2013. Temperature effects on pitfall catches of epigeal arthropods: a model and method for bias correction. *Journal of Applied Ecology*, 50: 181-189.
- Scheffer, S. J. 2000. Molecular evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Journal of Economic Entomology*, 93: 1146-1151.
- Scheffer, S. J., & Lewis, M. L. 2001. Two nuclear genes confirm mitochondrial evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Annals of the Entomological Society of America*, 94: 648-653.
- Scholtens, B., & Solis, M. 2015. Annotated check list of the Pyraloidea (Lepidoptera) of America North of Mexico. *ZooKeys*, 535: 1–136.
- Schowalter, T. D. 2016. Insect ecology: An ecosystem approach, fourth edition. Academic press, Amsterdam.
- SENAMHI. 2021. Datos / Descarga de datos Meteorológicos. Servicio Nacional de Meteorología e Hidrología del Perú. <https://www.senamhi.gob.pe/?p=descarga-datos-hidrometeorologicos>
- SENASA. 2022. Lista de plaguicidas agrícolas registrados en el Perú. Servicio Nacional de Sanidad Agraria. https://servicios.senasa.gob.pe/SIGIAWeb/sigia_consulta_producto.html
- Senior, L. J., & McEwen, P. K. 1998. Laboratory study of *Chrysoperla carnea* (Stephens) (Neuropt., Chrysopidae) predation on *Trialeurodes vaporariorum* (Westwood) (Hom., Aleyrodidae). *Journal of Applied Entomology*, 122: 99-101.
- Senior, L. J., & McEwen, P. K. 2007. The use of lacewings in biological control. In: McEwen, P., New, T., & Whittington, A. E. (Eds.). Lacewings in the crop environment. Cambridge University Press, New York, pp. 296–302.
- Serbina, L., Burckhardt, D., Birkhofer, K., Syfert, M., & Halbert, S. 2015. The potato pest *Russelliana solanicola* Tuthill (Hemiptera: Psylloidea): Taxonomy and host-plant patterns. *Zootaxa*, 4021: 33-62.
- Shivankar, S. B., Magar, S. B., Shinde, V. D., Yadav, R. G., & Patil, A. S. 2008. Field bio-efficacy of chemical, botanical and bio-pesticides against *Spodoptera litura* Fab. in sugar beet. *Annals of Plant Protection Sciences*, 16: 312-315.
- Shufran, K., & Puterka, G. 2011. DNA barcoding to identify all life stages of holocyclic cereal aphids (Hemiptera: Aphididae) on wheat and other Poaceae. *Annals of the Entomological Society of America*, 104: 39-42.

- Sidney, L. A., Bueno, V. H. P., Lins, Jr. J. C., Sampaio, M. V., & Silva, D. B. 2010. Larval competition between *Aphidius ervi* and *Praon volucre* (Hymenoptera: Braconidae: Aphidiinae) in *Macrosiphum euphorbiae* (Hemiptera: Aphididae). *Environmental Entomology*, 39: 1500-1505.
- SIEA. 2022. *Sistema Integrado de Estadística Agraria*. <https://siea.midagri.gob.pe/portal/estadisticas>
- Siegfried, B. (1993). Comparative toxicity of pyrethroid insecticides to terrestrial and aquatic insects. *Environmental Toxicology and Chemistry: An International Journal*, 12: 1683-1689.
- Sifuentes, E., Albújar, E., Contreras, S., León, C., Moreyra, J., & Santa María, J. 2016. Anuario estadístico de la producción agrícola y ganadera 2016. Sistema Integrado de Estadísticas Agrarias del Ministerio de agricultura y riego del Perú. <http://siea.minagri.gob.pe/siea/?q=publicaciones/anuario-de-produccion-pecuaria>
- Sinclair, B. J., Williams, C. M., & Terblanche, J. S. 2012. Variation in thermal performance among insect populations. *Physiological and Biochemical Zoology*, 85: 594-606.
- Singh, A., & Sharma, O. P. 2004. Integrated pest management for sustainable agriculture. In: BIRTHAL, P. S., & SHARMA, O. P. (Eds.). *Integrated pest management in Indian agriculture*. Proceedings 11, New Delhi, pp. 11–24.
- Slater, J. A. 1964. *A catalogue of the Lygaeidae of the world*. University of Connecticut Press.
- Soca, N. 2021. Fluctuación poblacional de Insectos fitófagos asociados al cultivo de quinua (*Chenopodium quinoa* Willd.) en La Molina [Master thesis]. Universidad Nacional Agraria La Molina, Lima, Peru.
- Solis, M. A. 2006. Key to selected Pyraloidea (Lepidoptera) larvae intercepted at US ports of entry: Revision of Pyraloidea in “Keys to some frequently intercepted Lepidopterous larvae” by Weisman 1986. <https://digitalcommons.unl.edu/systemtomologyusda/1/>
- Sosa-Zuniga, V., Brito, V., Fuentes, F., & Steinfort, U. 2017. Phenological growth stages of quinua (*Chenopodium quinoa*) based on the BBCH scale. *Annals of Applied Biology*, 171: 117-124.
- Soto, E., Mercado, W., Estrada, R., Díaz, F., & Díaz, G. 2015. El mercado y la producción de quinua en el Perú. Instituto Interamericano de Cooperación para la Agricultura.
- Souza, B., Vázquez, L. L., & Marucci, R. C. 2019. Natural enemies of insect pests in Neotropical agroecosystems: Biological control and functional biodiversity. Springer Nature, Switzerland.
- Sparks, T., & Nauen, R. 2015. IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*, 121: 122-128.
- Spencer, K. 1973. *Agromyzidae (Diptera) of economic importance (Vol. 9)*. Springer Science & Business Media, Dordrecht.
- Spencer, K. A. 1990. *Host Specialization in the World Agromyzidae (Diptera)*. Kluwer Academic Publishers, Dordrecht.
- Spomer, N., & Sheets, J. 2019. Chitin biosynthesis and inhibitors. In: Jeschke, P., Witschel, M., Krämer, W., & Schirmer, U. (Eds.). *Modern crop protection compounds*. Wiley-VCH, Weinheim, Germany, pp. 1067–1084.
- Stanley, J., & Preetha, G. 2016. *Pesticide toxicity to non-target organisms: Exposure, toxicity and risk assessment methodologies*. Springer, Berlin, Germany.

- Stary, P. 1973. A review of the *Aphidius* species (Hymenoptera: Aphidiidae) of Europe. *Annotationes Zoologicae et Botanicae, Bratislava*, 85: 1-85.
- Steill, J., & Meyer, J. 2003. The Rhopalidae of Florida. *Insect Classification Project*, 4: 1-23.
- Stejskal, V., Vendl, T., Li, Z., & Aulicky, R. 2019. Minimal thermal requirements for development and activity of stored product and food industry pests (Acari, Coleoptera, Lepidoptera, Psocoptera, Diptera and Blattodea): a review. *Insects*, 10: 149.
- Stern, V. M., Smith, R. F., van den Bosch, R., & Hagen, K. S. 1959. The integrated control concept. *Hilgardia*, 29: 81–101.
- Straneo, S. 1986. Sul genere *Blennidus* Motschulsky 1865 (Col. Carabidae, Pterostichini). *Bollettino Del Museo Regionale Di Scienze Naturali Di Torino*, 4: 369-393.
- Suttman, C. E., & Barrett, G. W. 1979. Effects of sevin on arthropods an agricultural and old-field plant community. *Ecology*, 60: 628–641.
- Takano, F., & Castro, N. 2007. Avifauna en el campus de la Universidad Nacional Agraria La Molina (UNALM), Lima-Perú. *Ecología Aplicada*, 6: 149-154.
- Tapia, M. 1979. La quinua y kañiwa, granos andinos. IICA-CIID, Bogotá, Colombia.
- Tavares, W. S., Cruz, I., Silva, R. B., Serrão, J. E., & Zanuncio, J. C. 2011. Prey consumption and development of *Chrysoperla externa* (Neuroptera: Chrysopidae) on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs and larvae and *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs. *Maydica*, 56: 283-289.
- Thompson, F., Rotheray, G., & Zumbado, M. 2010. Syrphidae (Flower flies). In: Brown, B., Borkent, A., & Cumming, J. (Eds.). *Manual of Central American Diptera*. NRC Research Press, Ottawa, Ontario, Canada, pp. 7637–7690.
- Thrupp, L. A. 2004. The importance of biodiversity in agroecosystems. *Journal of Crop Improvement*, 12: 315-337.
- Torres, J. B., & Bueno, A. D. F. 2018. Conservation biological control using selective insecticides— a valuable tool for IPM. *Biological Control*, 126: 53-64.
- Trichilo, P. J., & Leigh, T. F. 1988. Influence of resource quality on the reproductive fitness of flower thrips (Thysanoptera: Thripidae). *Annals of the Entomological Society of America*, 81: 64–70.
- Triplehorn, C. A., & Johnson, N. F. 2005. Borror and DeLong's introduction to the study of insects. Thompson Brooks/Cole, Belmont.
- Tylianakis, J. M., Tscharrntke, T., & Lewis, O. T. 2007. Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature*, 455: 202-205.
- Valoy, M., Bruno, M., Prado, F., & González, J. 2011. Insectos asociados a un cultivo de quinua en Amaicha del Valle, Tucumán, Argentina. *Acta Zoológica Lilloana*, 55: 16-22.
- Valoy, M., Reguilón, C., & Podazza, G. 2015. The potential of using natural enemies and chemical compounds in quinoa for biological control of insect pests. In: Murphy, K. S., & Matanguihan, J. (eds.). *Quinoa: Improvement and sustainable production*. John Wiley & Sons, New Jersey, pp. 63–86.

- van Emden, H. F. 2013. Handbook of agricultural entomology. John Wiley & Sons, Ltd, Oxford.
- van Emden, H. F., & Williams, G. 1974. Insect stability and diversity in agro-ecosystems. *Annual Review of Entomology*, 19: 455-475.
- van Lenteren, J. C. 2000. Success in biological control of arthropods by augmentation of natural enemies. In: Gurr, G., & Wratten, S. (Eds.). Biological control: Measures of success. Springer, Dordrecht, pp. 77–103.
- van Lenteren, J. C. 2012. The state of commercial augmentative biological control: Plenty of natural enemies, but a frustrating lack of uptake. *BioControl*, 57: 1-20.
- Varenhorst, A. J., & O’Neal, M. E. 2012. The response of natural enemies to selective insecticides applied to soybean. *Environmental Entomology*, 41: 1565-1574.
- Vasantharaj, B., & Ananthkrishnan, T. 2004. General and applied entomology, second edition. Tata McGraw-Hill, New Delhi.
- Vásquez, L. K. 2016. Determinación del ciclo de desarrollo, biológico, parámetros biológicos y comportamiento de *Nysius* sp. (Hemiptera: Lygaeidae) “chinche menor de la quinua” bajo condiciones de laboratorio. Santa Rita de Siguan. Arequipa [Bachelor thesis]. Universidad Nacional de San Agustín, Arequipa, Peru.
- Venzon, M., Martins, E. F., Batista, M. C., Botti, J. M. C., Andrade, F. P., & Barroso, A. M. 2021. Green lacewings and their role in pest management. Controle alternativo de pragas e doenças: Opção ou necessidade? Belo Horizonte: Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG).
- Vilca, K. 2010. Contribución al conocimiento de las especies de la familia Aphididae del Callejón del Huaylas – Áncash [Master thesis]. Universidad Nacional Agraria La Molina, Lima, Peru.
- Vilca, K., & Vergara, C. 2011. Los áfidos (Hemiptera: Aphididae) en El Callejón de Huaylas–Ancash, Perú. *Ecología Aplicada*, 10: 93-98.
- Vilca, S., Espinoza, E., & Vidal, A. 2015. Multiplicación de semilla de variedades y ecotipos de quinua en valle de majes-Arequipa. *Revista Investigaciones Altoandinas*, 17: 355-368.
- Vilímová, J., & Rohanová, M. 2010. The external morphology of eggs of three Rhopalidae species (Hemiptera: Heteroptera) with a review of the eggs of this family. *Acta Entomologica Musei Nationalis Pragae*, 50: 75-95.
- Villanueva, S. 1978. Determinación del “umbral económico” y “nivel crítico” de “kcona kcona” (*Scrobipalpula* sp.) en quinua (*Chenopodium quinoa* Willd.) [Bachelor thesis]. Universidad Nacional del Altiplano, Puno, Peru.
- Villena, G. 2011. Sistemas de cultivo de la quinua (*Chenopodium quinoa* Willd.) y su efecto en el rendimiento y calidad en condiciones de verano en La Molina [Bachelor thesis]. Universidad Nacional Agraria La Molina, Lima, Peru.
- Wagner, C. H., Cox, M., & Robles, J. L. B. 2016. Pesticide lock-in in small scale Peruvian agriculture. *Ecological Economics*, 129: 72-81.
- Wallner, W. 1987. Factors affecting insect population dynamics: Differences between outbreak and non-outbreak species. *Annual Review of Entomology*, 32: 317-340.

- Walsh-Dilley, M. 2016. Tensions of resilience: Collective property, individual gain and the emergent conflicts of the quinoa boom. *Resilience*, 4: 30-43.
- Wenninger, E., & Inouye, R. 2008. Insect community response to plant diversity and productivity in a sagebrush steppe ecosystem. *Journal of Arid Environments*, 72: 24-33.
- Wei, J.; Zou, L.; Kuang, R., & He, L. 2000. Influence of Leaf Tissue Structure on Host Feeding Selection by Pea Leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Zoological Studies*, 39: 295-300.
- Wheeler, A. G. 2016. *Liorhyssus hyalinus* (F.) (Hemiptera: Rhopalidae) in the western United States: New host records, host-plant range, and comments on use of the term "host plant". *Proceedings of the Entomological Society of Washington*, 118: 115-128.
- Wilby, A., & Thomas, M. B. 2002. Natural enemy diversity and pest control: Patterns of pest emergence with agricultural intensification. *Ecology Letters*, 5: 353-360.
- Wilcox, J., & Howland, A. F. 1955. Control of the pea leaf miner in southern California. *Journal of Economic Entomology*, 48: 579-581.
- Willott, S. (2001). Species accumulation curves and the measure of sampling effort. *Journal of Applied Ecology*, 38: 484-486.
- Wolfe, M. S. 2002. The role of functional biodiversity in managing pests and diseases in organic production systems. *The British Crop Protection Council Conference: Pests and diseases 1,2*: 531–538.
- Yábar, E., Gianoli, E., & Echegaray, E. 2002. Insect pests and natural enemies in two varieties of quinoa (*Chenopodium quinoa*) at Cusco, Peru. *Journal of Applied Entomology*, 126: 275-280.
- Youming, H., Xiongfei, P., Guangwen, L., & Minsheng, Y. 2001. Effect of chemical insecticides on the diversity of arthropods in vegetable fields. *Acta Ecologica Sinica*, 21: 1262-1268.
- Zhang, H., & Rojas, H. A. 2010. Teoría estadística: Aplicaciones y métodos. Facultad de Estadística, Universidad Santo Tomás. Bogotá, Colombia.
- Zhang, P., Zhang, X., Zhao, Y., Wei, Y., Mu, W., & Liu, F. 2016. Effects of imidacloprid and clothianidin seed treatments on wheat aphids and their natural enemies on winter wheat. *Pest Management Science*, 72: 1141-1149.
- Zhu, H., Peng, Y., & Wang, D. 2008. Effects of plant on insect diversity: A review. *Chinese Journal of Ecology*, 27: 2215-2221.
- Zhu, Y. C., Yao, J., Adamczyk, J., & Luttrell, R. 2017a. Feeding toxicity and impact of imidacloprid formulation and mixtures with six representative pesticides at residue concentrations on honey bee physiology (*Apis mellifera*). *PLoS One*, 12: e0178421.
- Zhu, Y. C., Yao, J., Adamczyk, J., & Luttrell, R. 2017b. Synergistic toxicity and physiological impact of imidacloprid alone and binary mixtures with seven representative pesticides on honey bee (*Apis mellifera*). *PLoS One*, 12: e0176837.

Annexes

Annex 1

Comparison of the nutritional value of quinoa, rice, barley, corn and wheat in terms of the minerals (ppm), vitamins (ppm) and amino acids (g amino acid/16 g of nitrogen).

	Quinoa	Rice	Barley	Corn	Wheat
<u>Minerals</u>					
Ca	1274	276	880	700	500
P	3869	2845	4200	4100	4700
Fe	120	37	50	21	50
K	6967	2120	5600	4400	8700
Mg	2700	-	1200	1400	1600
Na	115	120	200	900	115
Cu	37	-	8	-	7
Mn	75	-	16	-	49
Zn	48	51	15	-	14
<u>Vitamins</u>					
Niacin	10.7	57.3	58.3	-	47.5
Tamine (B1)	3.1	3.5	3.3	-	6
Riboflavin (B2)	39	0.6	1.3	-	1.4
Ascorbic acid (C)	49	-	-	-	-
Alpha-Tocopherol (E)	52.3	-	-	-	-
Carotene	5.3	-	3.7	-	0.1
<u>Amino acids</u>					
Arginine	7.3	6.9	4.8	4.2	4.5
Phenylamine	4	5	5.2	4.7	4.8
Histidine	3.2	2.1	2.2	2.6	2
Isoleucine	4.9	4.1	3.8	4	4.2
Leucine	6.6	8.2	7	12.5	6.8
Lysine	6	3.8	3.6	2.9	2.6
Methionine	2.3	2.2	1.7	2	1.4
Threonine	3.7	3.8	3.5	3.8	2.8
Tryptophane	0.9	1.1	1.4	0.7	1.2
Valine	4.5	6.1	5.5	5	4.4

Annex 2

Agroecological zones of quinoa production in Peru and the studied field sites (La Molina, San Lorenzo and Majes) located on the map of Peru.

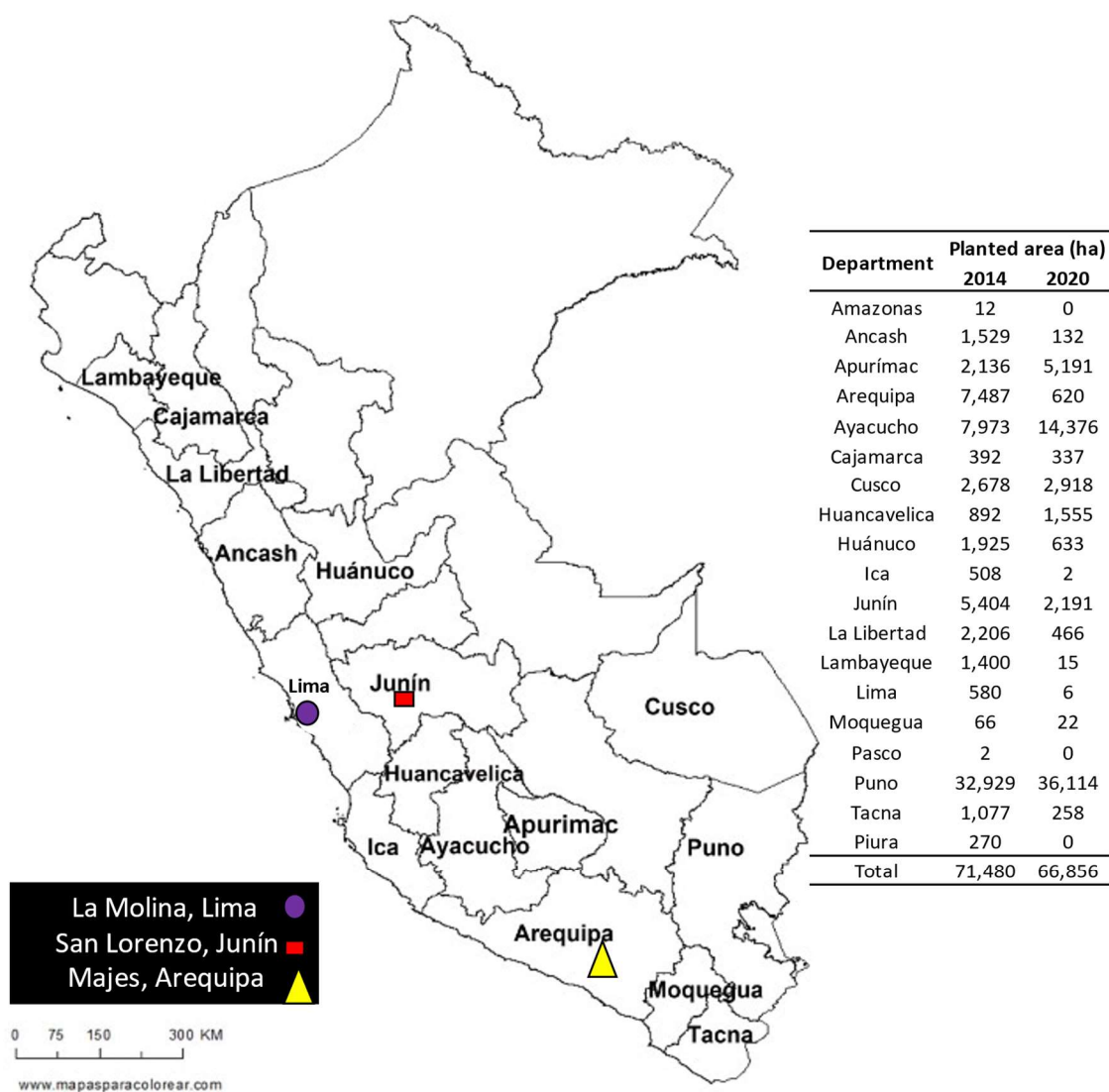


Source:

https://commons.wikimedia.org/wiki/File:Mapa_topogr%C3%A1fico_del_Per%C3%BA.png

Annex 3

Departments of Peru where quinoa has been cultivated in 2014 and 2020 and the studied field sites (chapters 3 and 4) located on the map of Peru.



Annex 4

Reported parasitoids from Peru whose host range includes pests of quinoa from the Andean and Coastal regions.

Order	Family	Parasitoid (genus/species)	Host		
Diptera	Tachinidae	<i>Prosopochaeta setosa</i>	<i>Copitarsia</i> spp.		
		<i>Patelloa similis</i>	<i>Copitarsia</i> spp.		
		<i>Incamiya</i> sp.	<i>Copitarsia</i> spp.		
		<i>Euphorocera peruviana</i>	<i>Copitarsia</i> spp., <i>Chloridea virescens</i>		
		<i>Winthemia</i> sp.	<i>Copitarsia</i> spp.		
		<i>Dolichostoma arequipae</i>	<i>Copitarsia</i> spp.		
		<i>Peleteria robusta</i>	<i>Copitarsia</i> spp.		
		<i>Meigenia mutabilis</i>	<i>Helicoverpa quinoa</i>		
		<i>Gymnosoma</i> sp.	<i>Helicoverpa quinoa</i>		
		<i>Phytomyptera</i> sp.	<i>Eurysacca melanocampta</i> , <i>E. quinoa</i>		
		<i>Dolichostoma</i> sp.	<i>Eurysacca melanocampta</i> , <i>E. quinoa</i>		
		<i>Gonia peruviana</i>	<i>Agrotis</i> spp.		
		<i>Gonia pallens</i>	<i>Agrotis</i> spp.		
		<i>Archytas marmoratus</i>	<i>Agrotis</i> spp., <i>Spodoptera</i> spp., <i>Chloridea virescens</i>		
		<i>Linnaemya compta</i>	<i>Agrotis</i> spp.		
		<i>Prosopochaeta fidelis</i>	<i>Agrotis</i> spp.		
		<i>Velardemyia ica</i>	<i>Agrotis</i> spp.		
		<i>Winthemia reliqua</i>	<i>Agrotis</i> spp., <i>Spodoptera</i> spp.		
		<i>Protogoniops</i> sp.	<i>Spodoptera</i> spp.		
		<i>Gonia</i> sp.	<i>Chloridea virescens</i>		
		<i>Hemilydella fasciata</i>	<i>Chloridea virescens</i>		
		<i>Eucelatoria australis</i>	<i>Spodoptera</i> spp., <i>Chloridea virescens</i>		
		<i>Eucelatoria digitata</i>	<i>Chrysodeixis includens</i> , <i>Chloridea virescens</i>		
		<i>Lespesia</i> sp.	<i>Chrysodeixis includens</i>		
		<i>Voria ruralis</i>	<i>Chrysodeixis includens</i>		
		<i>Leucostoma</i> sp.	<i>Liorhyssus hyalinus</i>		
		<i>Comatacta variegata</i>	<i>Spoladea recurvalis</i>		
			Sarcophagidae	<i>Sarcophaga</i> sp.	<i>Helicoverpa quinoa</i>
		Hymenoptera	Braconidae	<i>Apanteles elegans</i>	<i>Agrotis</i> spp.
				<i>Chenolus</i> sp.	<i>Agrotis</i> spp.
				<i>Meteorus chilensis</i>	<i>Agrotis</i> spp.
				<i>Cotesia marginiventris</i>	<i>Spodoptera</i> spp.
				<i>Chelonus insularis</i>	<i>Spodoptera</i> spp.
<i>Rogas</i> sp.	<i>Spodoptera</i> spp.				
<i>Apanteles</i> sp.	<i>Eurysacca melanocampta</i> , <i>E. quinoae</i>				
<i>Meteorus</i> sp.	<i>Eurysacca melanocampta</i> , <i>E. quinoae</i>				
<i>Microplitis</i> sp.	<i>Eurysacca melanocampta</i> , <i>E. quinoae</i>				
<i>Venanus kusikuylluræ</i>	<i>Eurysacca melanocampta</i> , <i>E. quinoae</i>				
<i>Aphidius matricariae</i>	<i>Macrosiphum euphorbiae</i> , <i>Myzus</i> sp.				
<i>Aphidius colemani</i>	<i>Macrosiphum euphorbiae</i> , <i>Myzus</i> sp.				
<i>Lysiphlebus testaceipes</i>	<i>Macrosiphum euphorbiae</i> , <i>Myzus</i> sp.				
<i>Praon volucre</i>	<i>Macrosiphum euphorbiae</i> , <i>Myzus</i> sp.				

Reported parasitoids from Peru whose host range includes pests of quinoa from the Andean and Coastal regions (continued).

Order	Family	Parasitoid (genus/species)	Host	
Hymenoptera	Encyrtidae	<i>Opius scraventris</i>	<i>Liriomyza huidobrensis</i>	
		<i>Opius</i> sp.	<i>Liriomyza huidobrensis</i>	
		<i>Copidosoma gelechia</i>	<i>Eurysacca melanocampta</i> , <i>E. quinoae</i>	
		<i>Copidosoma</i> sp.	<i>Eurysacca melanocampta</i> , <i>E. quinoae</i>	
	Eulophidae	<i>Litomastix truncantella</i>	<i>Chrysodeixis includens</i>	
		<i>Euplectrus platypenae</i>	<i>Chrysodeixis includens</i> , <i>Copitarsia</i> spp.	
		<i>Closterocerus cinctipennis</i>	<i>Liriomyza huidobrensis</i>	
		<i>Chrysocharis phytomyzae</i>	<i>Liriomyza huidobrensis</i>	
		<i>Chrysocharis</i> sp.	<i>Liriomyza huidobrensis</i>	
		<i>Derostenus</i> sp.	<i>Liriomyza huidobrensis</i>	
		<i>Diglyphus websteri</i>	<i>Liriomyza huidobrensis</i>	
		<i>Diglyphus begini</i>	<i>Liriomyza huidobrensis</i>	
		<i>Zagrammosoma multilineatum</i>	<i>Liriomyza huidobrensis</i>	
		<i>Agromyzophagus</i> sp.	<i>Liriomyza huidobrensis</i>	
		Ichneumonidae	<i>Enicospilus</i> sp.	<i>Agrotis</i> spp., <i>Chloridea virescens</i>
			<i>Ophion</i> sp.	<i>Agrotis</i> spp.
	<i>Coccygomimus</i> sp.		<i>Agrotis</i> spp.	
	<i>Enicospilus merdarius</i>		<i>Spodoptera</i> spp.	
	<i>Campoletis flavicincta</i>		<i>Spodoptera</i> spp., <i>Chloridea virescens</i>	
	<i>Campoletis curvicauda</i>		<i>Spodoptera</i> spp.	
	<i>Devorgilla peruviana</i>		<i>Chloridea virescens</i>	
	<i>Netelia</i> sp.		<i>Copitarsia</i> spp.	
	<i>Hyposoter</i> sp.		<i>Copitarsia</i> spp.	
	<i>Diadegma</i> sp.		<i>Eurysacca melanocampta</i> , <i>E. quinoae</i>	
	<i>Deleboea</i> sp.		<i>Eurysacca melanocampta</i> , <i>E. quinoae</i>	
	<i>Venturia</i> sp.		<i>Eurysacca melanocampta</i> , <i>E. quinoae</i>	

Annex 5

Number of morphospecies per family found at the field sites in San Lorenzo, Majes and La Molina throughout the crop phenology of quinoa.

Order	Family	No. of morphospecies			
		San Lorenzo	Majes	La Molina	
Coleoptera	Anthicidae	0	1	3	
	Bostrichidae	0	0	1	
	Carabidae	4	2	4	
	Chrysomelidae	4	0	1	
	Coccinellidae	3	1	2	
	Curculionidae	2	0	1	
	Dytiscidae	1	0	0	
	Elateridae	1	0	1	
	Latriidae	1	2	2	
	Melyridae	1	0	0	
	Mycetophagidae	0	1	0	
	Nitidulidae	2	2	2	
	Ptilidae	3	0	1	
	Scarabaeidae	0	2	2	
	Staphylinidae	32	12	8	
	Tenebrionidae	3	4	2	
	Undetermined	1	5	0	
	Diptera	Agromyzidae	3	1	1
		Anthomyidae	9	4	6
Asilidae		0	0	2	
Asteiidae		0	0	1	
Bibionidae		1	0	0	
Calliphoridae		2	0	3	
Cecidomyiidae		16	0	1	
Ceratopogonidae		1	0	3	
Chironomidae		8	1	1	
Chloropidae		4	1	4	
Cypselosomatidae		7	0	0	
Diastatidae		1	0	0	
Dolichopodidae		4	0	3	
Drosophilidae		2	2	2	
Empididae		4	1	0	
Ephydriidae		1	1	0	
Keroplastidae		1	1	0	
Milichiidae		1	0	0	
Muscidae		1	1	1	
Mycetophilidae		1	0	0	
Phoridae		1	1	2	
Psychodidae		0	1	0	
Pipunculidae		1	0	0	
Sciariidae		17	2	1	
Sarcophagidae		7	2	4	
Scatopsidae		1	1	0	
Syrphidae		6	0	2	

Number of morphospecies per family found at the field sites in San Lorenzo, Majes and La Molina throughout the crop phenology of quinoa (continued).

Order	Family	No. of morphospecies			
		San Lorenzo	Majes	La Molina	
Diptera	Tachinidae	9	0	2	
	Tipulidae	1	0	0	
	Ulidiidae	1	0	0	
Ephemeroptera	Undetermined	2	4	2	
	Undetermined	1	0	0	
Hemiptera	Anthocoridae	3	1	0	
	Aleyrodidae	0	1	0	
	Aphididae	4	3	7	
	Cicadellidae	22	12	10	
	Cixiidae	0	0	1	
	Cydnidae	1	0	0	
	Delphacidae	1	1	0	
	Geocoridae	0	1	0	
	Lygaeidae	0	1	1	
	Miridae	4	2	2	
	Nabidae	0	1	1	
	Psyllidae	1	1	0	
	Rhopalidae	1	1	1	
	Triozidae	1	0	1	
	Undetermined	0	1	0	
	Hymenoptera	Apidae	1	0	1
		Braconidae	8	2	3
Encyrtidae		2	2	1	
Eulophidae		3	0	7	
Figitidae		2	2	0	
Formicidae		4	1	9	
Halictidae		1	0	1	
Ichneumonidae		10	0	2	
Mymaridae		14	2	5	
Pompilidae		2	0	0	
Pteromalidae		2	1	2	
Scelionidae		9	0	5	
Tiphiidae		0	2	0	
Lepidoptera	Trichogrammatidae	1	0	0	
	Undetermined	18	2	17	
	Crambidae	5	0	0	
	Gelechiidae	4	1	1	
	Noctuidae	2	1	1	
Neuroptera	Undetermined	0	3	0	
	Chrysopidae	0	2	0	
Orthoptera	Hemerobiidae	1	0	0	
	Gryllidae	1	0	1	
Psocoptera	Undetermined	0	2	1	
Thysanoptera	Thripidae	1	1	2	
Total number of morphospecies		301	106	154	

Acknowledgements

I would like to express my special gratitude to my advisors, Prof. Dr. Patrick De Clercq and Prof. Dr. Eduardo de la Peña, for their invaluable supervision, support and tutelage during the course of my PhD research. Their immense knowledge and plentiful experience have always been my motivation to grow as a scientist, throughout the years that took my PhD thesis.

Thanks to the members of the examination committee for their comments and suggestions that made me to improve my dissertation.

I acknowledge VLIR-UOS/UNALM, for funding this study and professor Eddie Schrevens of KU Leuven for his unwavering support. Thanks to the VLIR members from Belgium: Tupac Calfat and Nancy Terryn and to the VLIR staff in La Molina: Jenny Paz, Luz María López and Vannesa León for all your help provided.

Thank you to the members of the technical staff in the Laboratory of Agrozoology: Agnes, Bjorn, Leen, Stephanie and Rik, for the kindness and friendly assistance during my stance in Belgium.

Thank Wim Jonckheere for your patience when teaching all procedures related to PCR.

Guy De Groote and Anne-Marie Bonamie, thank you for your kindness during my stance in Ghent.

Thank you to my colleagues at Museum of Entomology, Klaus Raven Büller, from Peru for their invaluable advice and motivation: the professors Alexander Rodríguez, Guillermo Sánchez, Norma Mujica and in particular to Clorinda Vergara for the facilities, permits and her teachings; my friends Yony Callohuari, Nelly Socca, and a special thanks to Carmen Livia for give me a hand when always I needed, and for your encouragement during all these endless years that took my PhD thesis and for your company during my lab activities, I do appreciate all those moments.

Thanks Cristian Ocaña, for your help during my samplings in Arequipa, and in La Molina, for your assistance and company during my lab activities and also during my field inspections in La Molina. This was an arduous job that I couldn't manage alone. You will always have my immense gratitude for this.

Thank you to the professors Luz Gomez and Jorge Jimenez from the Cereal and Native Grains Program, for the facilities and permissions. Gracias al personal del Programa de Cereales y Granos Nativos que colaboró intensamente en las actividades de campo: Cesar Augusto Inga Ore, Marcelina Flores Quito, Osvaldo Raúl Rosas Arteaga, Carmen Flores Choquechahua, Erodita

Tineo Crisanto, Hugo Ccente Quispe, William Wilfredo Romero Pezo, Ing. José Santiago Falconi Palomino, Ing. Katherine Ruth Argumedo Gonzales, Ing. María Corina Ponce Encinas. Un agradecimiento muy especial a Ruth Teofila Paucar Serda, secretaria del programa, quien ha visto mi desarrollo profesional desde estudiante de pregrado, y quien me ha motivado siempre a seguir creciendo profesionalmente; gracias Ruthie por tu amistad y por el invaluable apoyo que me has brindado durante todos estos años, y por el cual estaré eternamente agradecido.

Gracias a mis hermanas Erika, Yadira y Marianella, a mi madre Carmen, y mi cuñado Oscar, por su motivación y sus oraciones para que todo me salga bien. Su apoyo emocional fue fundamental para poder concluir este largo proceso. Gracias sobrinos, Fernanda, Sebastián y Naira!

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Publications

Iannacone, J. A., Ayala, H., Alvarino, L., Espinal, C. P., Villegas, W., Alomia, J., Santos S., Nolazco N. & **Cruces, L.** 2014. Riesgo ecotoxicológico acuático y terrestre del bioplaguicida catahua, *Hura crepitans* (Euphorbiaceae). *Revista de toxicología*, 31: 50-62.

Cruces, L., & Brailovsky, H. 2014. A new species of *Petersitocoroides* Brailovsky (Hemiptera: Heteroptera: Coreidae: Coreini) from Peru. *Zootaxa*, 3847: 590-594.

Cruces, L., Vergara, C., & Brailovsky, H. 2014. Nuevos registros de especies de la tribu Coreini (Hemiptera: Heteroptera: Coreidae) para Perú. *Revista Peruana de Entomología*, 49: 161-169.

Cruces, L., & Vergara, C. 2015. Distribución de las especies de ocho géneros de la tribu Coreini (Heteroptera: Coreidae) en Perú. *Ecología Aplicada*, 14: 41-54.

Cruces, L., Callohuari, Y., & Carrera, C. 2016. Quinoa: Manejo integrado de plagas. Estrategias en el cultivo de quinoa para fortalecer el sistema agroalimentario en la zona andina. Organización de las naciones unidas para la alimentación y la agricultura, Santiago, Chile. (online). <http://www.fao.org/3/a-i6038s.pdf>

Cruces, L., de la Peña, E., & De Clercq, P. 2020. Insect diversity associated with quinoa (*Chenopodium quinoa* Willd.) in three altitudinal production zones of Peru. *International Journal of Tropical Insect Science*, 40: 955-968.

Cruces, L., de la Peña, E. & De Clercq, P. 2020. Seasonal phenology of the major insect pests of quinoa (*Chenopodium quinoa* Willd.) and their natural enemies in a traditional zone and two new production zones of Peru. *Agriculture*, 10: 644.

Livia, C., Sánchez, G., & **Cruces, L.** 2020. Diversidad de insectos del suelo asociados al cultivo de maíz (*Zea mays* L.) en La Molina/Lima/Perú. *Ecología Aplicada*, 19: 57-64.

Cruces, L., de la Peña, E., & De Clercq, P. 2021. Field evaluation of cypermethrin, imidacloprid, teflubenzuron and emamectin benzoate against pests of quinoa (*Chenopodium quinoa* Willd.) and their side effects on non-target species. *Plants*, 10: 1788.

Oral Presentations

Cruces, L., de la Peña, E., & De Clercq, P. 2020. Insect diversity associated with quinoa (*Chenopodium quinoa* Willd.) in three altitudinal production zones of Peru. Entomology 2020, ESA's Virtual Annual Meeting, The Entomological Society of America (ESA). November 11-25, 2020.

Cruces, L., de la Peña, E., & De Clercq, P. 2021. Field evaluation of insecticides against pests of quinoa and their side effects on non-target species. Entomology 2021, The Entomological Society of America (ESA). October 31-November 3, 2021, Denver, Colorado.

Poster Presentations

Cruces, L., de la Peña, E., & De Clercq, P. 2021. Seasonal phenology of the major insect pests of quinoa (*Chenopodium quinoa* Willd.) and their natural enemies in a traditional zone and two new production zones of Peru. 72nd International Symposium on Crop Protection, virtual edition, May 18, 2021, Ghent, Belgium.