# 1 Biological Control in Latin America and the Caribbean: Information Sources, Organizations, Types and Approaches in Biological Control

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**Abstract**

Biological control with arthropod natural enemies and microbial control agents has been applied since the year 1895 in Latin America and the Caribbean and is currently used on a very large scale. Sources about the history and current situation of biocontrol in this region were not easy to trace and are, therefore, presented in this chapter. Next, organizations working on biocontrol in this region are listed. This is followed by a description of natural, conservation, classical and augmentative biocontrol with some regional examples. Then, an approach to find, evaluate and use biocontrol agents is sketched, as guidance for research projects. Often, tens to a hundred biocontrol candidates are found in association with a pest. A well organized research approach using evaluation criteria allows for quick exclusion of unsuitable or problematic candidate species. Biocontrol research has limited funding and early elimination of poor candidates results in spending more money on promising candidates. Regulations concerning import and release of agents that have been implemented during the past 30 years are summarized. Effects of these regulations are that prospecting for exotic natural enemies is now very difficult and that fewer new biocontrol agents have become available. Finally, the structure of the book is explained.

## 1.1 Introduction

Biological control (biocontrol) is, simply said, the use of an organism to reduce the population density of another organism. It is the most successful, most cost effective and environmentally safest system for pest, disease and weed management (Bale *et al.*, 2008). It is nature's own way to keep numbers of pest organisms at low levels. Biocontrol is present in all ecosystems, both natural and man-made. The result of natural biocontrol is that the earth is green and that plants can produce sufficient biomass to sustain other forms of life. Without biocontrol, the production of energy by plants would be a tiny fraction of what is generated currently.

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# 7 Biological Control in Chile

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**Abstract**
The first introduction of a predator of olive black scale in 1903 marked the start of biocontrol in Chile. In 1915 the Ministry of Agriculture began a programme of introduction of beneficial organisms and since then approximately 200 species of beneficial insects have been introduced into the country. Many complete and permanent successes were obtained in the period 1900–1969 and examples are control of: olive black scale; scales in grapevine, citrus, berries, avocado and other fruit; mealybugs in citrus, grapevine, avocado and other fruit; and woolly apple aphid in apple. The use of microbial control agents started in the 1950s and numerous microorganisms have since been collected, identified and mass produced to control pests and diseases. Weed biocontrol began in Chile in the 1950s with successful classical biocontrol of St John's wort with a phytophagous weevil, followed in the 1970s by using a plant pathogenic fungus for control of *Rubus ulmifolius* (zarzamora) and in the 1980s–1990s by releasing phytophagous weevils, moths and mites for control of gorse weed. An important large project executed during the 1970s and 1980s resulted in classical biocontrol of several virus-transmitting aphid species on thousands of hectares of wheat and barley. In forestry, parasitoids have been introduced for classical biocontrol of European pine shoot moth and of the sirex wood wasp in pine, and the eucalyptus psyllid in eucalyptus on vast areas. Currently, there are many commercial and governmental research initiatives for predators, parasitoids, entomopathogenic fungi and nematodes, and with bacteria and fungi for pest and disease control.

## 7.1 Introduction

Chile has an estimated population of almost 17,800,000 (July 2017) and its main agricultural products are grapes, wine, apples, pears, onions, wheat, maize, oats, peaches, garlic, asparagus, beans, beef, poultry, wool, fish and timber (CIA, 2017). According to Schick *et al.* (2017, pp. 192, 193 and 198):

> ... the advent of export agriculture created two types of agriculture: one dedicated to supplying food for the local population, developed by Peasant Family Agriculture, in small, low-tech plots of land; and commercial agriculture, with a great deal of technology and significant foreign investment, designed to produce for world markets. ... mainland Chile has an area of 75.6 million hectares (ha), 51.7 million of which are suitable for silvo-agriculture and 35.5 million of which are used for agricultural livestock raising or forestry. However, due to geographical and economic factors, the area under cultivation currently stands at just 2.12 million ha. This area is distributed among 1,303,210 ha of annual and permanent crops, 401,018 ha of sown fields and 419,714 ha of fallow land. A total of 17,070,776 ha are covered by native forest and bushes; 12,549,478 by natural meadows; 2,707,461 by forest plantations, and 1,062,352 by improved pastures. Of the 1.3 million ha with annual and permanent crops, 704,575 ha are used for annual crops (wheat, corn, oats, potato and raps), 296,587 ha for fruit trees (table vines, apple, avocado, walnut, cherry), 137,593 ha for wine-grape vines and 78.072 ha for vegetables (corn, lettuce, tomato, onion, marrow). As for meat production, poultry accounts for the largest share, ... next pork ..., beef ..., lamb ... and horsemeat. ... The Chilean salmon aquaculture industry is the now second largest export sector in the country and the world's second largest salmon producer after Norway.

## 7.2 History of Biological Control in Chile

Many agricultural and forest pests are exotic to Chile and have been introduced into the country by different means, including tourism, machinery packing, ships, aircraft and as a result of self-dispersion of some species. Once introduced, they usually do not meet any natural enemies in the country and their populations can multiply easily.

### 7.2.1 Period 1880–1969

After the initiative of a farmer who imported a natural enemy of olive black scale *Saissetia oleae* (Olivier) into Chile in 1903, the Ministry of Agriculture began a programme to introduce beneficial insects in 1915. Since then, classical biocontrol has been the dominant practice, with approximately 200 species of beneficial insects introduced into Chile to control different pests. Many of the natural enemies have been successfully established and they controlled pests without the farmers realizing it. The main sources of the beneficial insects introduced into Chile are the USA, Peru, Canada, Germany, England and Argentina (Rojas, 2005). From the 1930s, Chile also became an exporter of biocontrol agents, resulting in successes worldwide in controlling mosquitoes, aphids, scales and mites, among others. The use of microbial agents for biocontrol started in the 1950s. Numerous microorganisms have subsequently been used to control pests and diseases, some of which are commercialized for use in integrated pest management (IPM) or organic production.

### *Biological control of agricultural pests with arthropod natural enemies*

Biocontrol of pests in Chile has a long history and started in 1903 with the introduction of *Rhyzobius ventralis* (Erichson), a coccinellid predator of eggs and nymphs of the olive black scale, by farmer T. Schneider. This pest had been present in Chile since 1868. Between 1931 and 1946, natural enemies of *S. oleae* of the genera *Coccophagus*, *Lecanobius*, *Metaphycus* and *Scutellista* were introduced from the USA and Peru. The parasitoid *Metaphycus helvolus* (Compere) was reintroduced in 1951 by the La Cruz National Insectarium (now La Cruz Regional Research Center of the Instituto de Investigaciones Agropecuarias (INIA)) and this time the parasitoid successfully established and dispersed (Zuñiga, 1986). To this day, *M. helvolus* is one of the most effective and important natural enemies of *S. oleae* (Rojas, 2005). The different natural enemies introduced for control of *S. oleae* in Chile have also controlled other pests with similar characteristics (Table 7.1).

Other important pests in Chile are mealybugs, because they are considered quarantine pests by many countries importing Chilean fruit. Species of the genera *Pseudococcus* and *Planococcus* establish colonies on fruits, leaves, stems, trunks and roots of many hosts. The weakening caused by these sap-feeding insects can be serious and in some cases mealybugs kill the plant. Other deleterious effects arise from toxin production, virus transmission and honeydew secretion. Failures in mealybug control cause rejection of fruit in the inspecting ports or importing countries and consequently important economic losses. Biocontrol of these species started in Chile in 1931 when the predator *Cryptolaemus montrouzieri* (Mulsant) and the parasitoid *Leptomastidea abnormis* (Girault) were introduced. The La Cruz National Insectarium was founded in 1939 and the first natural enemy it produced was *C. montrouzieri*. New introductions of *C. montrouzieri* from the USA occurred until 1996. Between 1951 and 1958, four other parasitoids were introduced. Parasitoids of the most common mealybug species are listed in Table 7.2. *Coccophagus gurneyi* (Compere) and *C. montrouzieri* have been the most effective white mealybug natural enemies.

**Table 7.1.** Pests controlled by natural enemies originally introduced to control *Saissetia oleae* (retrieved from González and Rojas, 1966; Rojas, 2005; Ripa and Droguett, 2008).

| Natural enemies | Pests | Main host plants |
|---|---|---|
| *Coccophagus caridei*, *Metaphycus flavus*, *M. helvolus* | *Ceroplastes sinensis* (Del Guercio), Chinese wax scale | Grapevine |
| | *Ceroplastes cirripediformis* (Comstock), Florida wax scale | Orange, lemon, mandarin, grapefruit, cherry, plum, lucuma |
| | *Parthenolecanium corni* (Bouché), European fruit lecanium scale | Grapevines, chestnut, gooseberry, sarsaparrilla (*Smilax* sp.), cranberry |
| | *Parthenolecanium persicae* (Fabricius), European peach scale | Peach, grapevine |
| | *Pulvinaria mesembryanthemi* Vallot, Soft scale | Avocado |
| *C. caridei*, *M. flavus*, *M. helvolus*, *M. stanleyi* | *Coccus hesperidum* (Linnaeus), Florida soft scale | Orange, lemon, mandarin, grapefruit, raspberry, blueberry, guava |
| | *Protopulvinaria pyriformis* (Cockerell), Pyriform scale | Avocado, lucuma, guava, orange |
| | *Saissetia coffeae* (Walker), Hemispherical soft scale | Lemon, grapefruit, orange, olive, lucuma, mango, guave |

**Table 7.2.** Parasitoids introduced into Chile to control mealybugs (retrieved from Ripa and Rodríguez, 1999; Rojas, 2005; Ripa *et al*., 2008).

| Parasitoids | Pests | Main host plants |
|---|---|---|
| *Leptomastidea abnormis* (Girault), *L. dactylopii* (Howard), *Pauridia peregrina* (Timberlake), *Allotropa citri* (Muesebeck), *Anagyrus pseudococci* (Girault), *Pseudophycus perdignus* (Compere) | *Planococcus citri* (Risso) | Lemon, mandarin, orange, grapefruit, persimmon, pomegranate, cherimoya, guava, mango |
| *Coccophagus gurneyi* (Compere), *Tetracnemus pretiosus* (Timberlake) | *Pseudococcus calceolariae,* (Maskell) | Lemon, mandarin, orange, pear, grapefruit, blueberry, persimmon, cherimoya, plum, peach, quince, avocado, raspberry, sarsaparilla |
| | *Pseudococcus longispinus* (Targioni and Tozzetti) | As above, plus guava, sour cherry, lucuma, mango, apple, passion fruit, loquat, olive, grapevine |
| *Leptomastix epona* (Noyes), *Pseudaphycus flavidulus* (Bréthes) | *Pseudococcus viburni* (Maskell) | Alfalfa, persimmon, cherry, plum, citrus, raspberry, chickpea, lentil, apple, blackberry, nectarine, loquat, potato, melon pear, pear, radish, grapevine, sarsaparilla |

The woolly apple aphid *Eriosoma lanigerum* (Hausmann) is distributed throughout the apple orchard area in Chile and may cause serious damage. In the 1920s, this pest caused the death of a large number of trees, which led producers to abandon cultivation of apple. To control the pest, the parasitoid *Aphelinus mali* (Haldeman) was introduced from Uruguay in 1922 and had established by 1927 (Howard, 1929). The parasitoid adapted quickly to the country's conditions and re-energized the then weakened apple industry.

#### *Microbial control of agricultural and forest pests*

The first report about the use of entomopathogenic fungi (EPF) in Chile dates back to the 1950s when Dutky (1957) studied the effect of a pathogen of the beetle *Hylamorpha elegans* (Burmeister), which occurred in the southern regions of Chile and was a primary pest of wheat and pastures. The first report on biocontrol with entomopathogenic nematodes (EPN) also dates back to the 1950s, when *Steinernema* sp. (strain DD-136) was used for control of several soil pests, including larvae of coleopterans (*H. elegans, Pantomorus cervinus* (Boh.) (Curculionidae)) and lepidopterans (*Dalaca noctuides* Pfitzner, *Agrotys* sp.).

#### *Biological control of weeds*

Weed biocontrol began in Chile in the 1950s by introducing the beetles *Chrysolina hyperici* (Foster) and *Chrysolina quadrigemina* (Suffrian), which are specific to St John's wort (*Hypericum perforatum* L.). These species were successfully released and established in the country (Julien and Griffiths, 1998).

### 7.2.2 Period 1970–2000

#### *Biological control of agricultural pests with arthropod natural enemies*

One of the most successful cases of biocontrol in Chile was the programme to control cereal aphids. Until the beginning of the 1970s, wheat (*Triticum aestivum* L.) only showed low aphid densities not causing major problems. For still unknown reasons, several aphid species increased dramatically and began to cause serious yield losses to this Chilean staple food. In 1974 chemical insecticides were first applied on more than 120,000 ha of wheat (Zúñiga, 1986). In addition to the direct damage, the aphids transmitted viruses, such as the barley yellow dwarf virus (BYDV). Herrera and Quiroz (1988) showed that BYDV was able to reduce wheat yield by 10%, resulting in a yield loss of 80% in combination with direct damage caused by the aphids. The most damaging aphids were the pale green grass aphid *Metopolophium dirhodum* (Walker) and the grain aphid *Sitobion avenae* (Fabricius). The first attempts to control these aphids with beneficials go back to 1973 with introductions of parasitoids from Czechoslovakia and some predators, mainly coccinellids, from Canada, which had established in 1975.

In 1976, the Chilean government signed agreements with the Food and Agriculture Organization of the United Nations (FAO) and the United Nations Development Programme (UNDP) to bring expert consultants from California, through a National Biological Control of Aphids Program, focused on wheat and barley. Results of this programme were the implementation of new laboratory equipment at INIA, training of Chilean professionals and reliance on the support of international consultants, all of which contributed to the success achieved in biocontrol of wheat and barley aphids. Several parasitoids were brought to Chile, released and spread across the country; the aphid population was crushed and they never recovered for the next 40 years, saving a great amount of money for growers and consumers (Zúñiga, 1986). In addition, almost 300,000 ha per year were not sprayed with insecticides, avoiding environmental damage. In the mid-1980s, a new and dangerous aphid was accidentally introduced into Chile: the Russian wheat aphid *Diuraphis noxia* (Mordvilko). The same parasitoids introduced a decade earlier for control of other aphids also successfully controlled the Russian wheat aphid (Norambuena and Gerding, 1990; Rojas, 2005).

Apple orchards occupy more than 37,000 ha in Chile (ODEPA, 2016). *Cydia pomonella* (Linnaeus) causes important economic losses in apple, pear and nut orchards. Biocontrol of this pest was studied by using parasitoids of the genera *Trichogramma*, *Mastrus* and *Ascogaster* (Devotto *et al.*, 2010; Zaviezo and Mills, 2001). Torres and Gerding (2000) evaluated the parasitization efficiency of five species of *Trichogramma* and concluded that *T. cacoeciae* and *Trichogramma* sp. 'Cato' were efficient biocontrol agents of *C. pomonella*. Unfortunately, the apple industry in Chile still relies on chemical insecticides to control *C. pomonella*, instead of biocontrol.

### *Microbial control of agricultural pests*

The Universidad Austral de Chile conducted studies in the 1970s to identify the different species of the genus *Entomophthora* existing in the country and the insects for which infection had been reported. They concluded that five species of this genus infected insects located between Biobío and Los Ríos regions (Aruta *et al.*, 1974). In the same decade, studies were carried out with EPF as antagonists of insect larvae of the family Scarabaeidae present in grasslands. In the 1980s Ripa and Rodríguez (1989) found that eight strains of *Metarhizium anisopliae* (Metschnikoff) Sorokin exhibited high virulence in *Naupactus xanthographus* (Germar) larvae. At that time the development of biocontrol with EPF did not yet meet the standards of other countries where commercial formulations were already being developed. In addition to the work conducted by the Universidad Austral de Chile, INIA established a collection of microbial agents as a result of sampling carried out throughout the country. The collection started in 1996 with native strains that were isolated by the Insect Pathology Program of the Regional Research Center INIA-Quilamapu, Chillán. More than 500 accessions of EPF from different latitudes were incorporated between 1997 and 2000. Among the genera that make up this collection, *Metarhizium* and *Beauveria* are the most important. At the end of this decade, strains of *M. anisopliae* were evaluated against *Otiorhynchus sulcatus* (Fabricius) and all strains were pathogenic to varying degrees and depending on fungal spore concentrations (Gerding *et al.*, 2000). Strains of *Beauveria bassiana* (Balsamo) Vuillemin and *M. anisopliae* from the INIA collection were evaluated in 2000 as pathogens of *Aegorhinus superciliosus* (Guérin), *Asynonychus (Naupactus) cervinus* (Boheman) and *O. sulcatus,* which are considered to this day as economically important pests, mainly of berry fruits. Some of the evaluated strains showed high pathogenicity levels on insects in laboratory trials. Two of these strains are commercial products and are used in berry crops.

The first tests of bacteria for insect control started in 1996, when the effectiveness of two

*Bacillus thuringiensis* (Berliner) formulations was evaluated. The concentrations recommended by the manufacturers of Dipel and Javelin were effective to control *Helicoverpa zea* (Boddie). Assays were also carried out to evaluate the control effect of four *B. thuringiensis* strains against the European pine shoot moth *Rhyacionia buoliana* (Denis & Schiffermüller) (Huerta and Cogollor, 1995).

In the 1980s, nematodes of the family Rhabditidae (not identified at species level) for control of *N. xanthographus* larvae were evaluated; later the presence of saprophytic nematodes of the genus *Caenorhabditis* was noted in larvae of this same pest (Ripa, 1992). At the end of the 1980s, Jiménez *et al.* (1989) found that six out of seven species of Lepidoptera were susceptible to *Steinernema carpocapsae* (Weiser). In the 1990s the EPN *Pellioditis pellio* (Schneider) was evaluated for control of the native horsefly *Scaptia lata* (Guérin-Méneville). Nematodes of the family Mermithidae were found in *Procalus mutans* (Blanchard) and *P. reduplicatus* (Bechyné) beetles. Further, native EPNs associated with their symbiont bacteria belonging to the INIA collection were evaluated in the laboratory against *Deroceras reticulatum* (Müller), *A. superciliosus* and *A. cervinus* and promising results were obtained. The INIA collection contained 34 accessions by 2000, with *Heterorhabditis* and *Steinernema* as the most common genera.

### *Biological control of forest pests*

The forestry sector is very important to the Chilean economy and comprises 2,426,772 ha, of which 59% is *Pinus radiatea* D. Don and 34% *Eucalyptus* spp. (INFOR, 2016). One of the most important pests affecting pine nationwide was *R. buoliana*. The Chilean quarantine authority Servicio Agricola y Ganadero (SAG), INIA, the National Forest Corporation (Corporación Nacional Forestal) (CONAF) and several private forestry companies launched a programme to introduce natural enemies to control this pest. The Biological Control Center for the pine moth was implemented in the Regional Research Center INIA-Remehue in 1987 and the specific parasitoid *Orgilus obscurator* (Nees) was introduced from Europe that same year (Ide *et al.*, 2007). After a few years, this parasitoid reduced the moth outbreak below the economic damage threshold throughout the pine production area. The large forestry companies founded CPF S.A. (Forestry Pest Control Company) for *R. buoliana* control in the large private plantations, while CONAF provides consultancy, parasitized *R. buoliana* larvae and *O. obscurator* adults to small and medium-sized forest owners. The Regional Research Center INIA-Quilamapu started to mass produce and release the egg parasitoid *Trichogramma nerudai* (Pintureau & Gerding) in 1994 to further reduce moth outbreaks.

Another important forestry pest is the eucalyptus psyllid *Ctenarytaina eucalypti* (Maskell), which was detected in *Eucalyptus* spp. outbreaks in the Arica and Parinacota region in 1999. It spread rapidly over plantations throughout the country, causing damage as dehydration and death of primary shoots. To control this psyllid, INIA, SAG and CPF S.A. introduced the parasitoid *Psyllaephagus pilosus* (Noyes) from France and Peru in 2001. The parasitoid established and studies carried out by Rodríguez and Saiz (2006) demonstrated that parasitism of *C. eucalypti* was greater than 80%, especially during periods of maximum psyllid density.

### *Weed control with arthropod natural enemies and microbial agents*

*Ulex europaeus* L. or gorse, known as *espinillo* in Chile, is an important weed in agriculture and forestry. *Apion ulicis* (Foster), a seed-consuming weevil of gorse, was introduced in 1976, but results were disappointing (Norambuena and Piper, 2000). In the 1980s, the gorse moth *Agonopterix ulicetella* (Stainton), a defoliator that specifically attacks this weed, was introduced. New populations of this defoliator were introduced from the USA and England in the 1990s. After an intensive selection process, introduction, breeding and determination of its specificity, SAG authorized the release of founding populations in Chile in 1997. In 1996, *A. ulicetella* and the mite *Tetranychus lintearius* (Dufour) were introduced from Hawaii, as well as another ecotype of the same mite from Portugal. Martínez *et al.* (2000) concluded that *A. ulicetella* was not a risk for plant species that are different from their natural host after field release.

*Rubus ulmifolius* (Schott.), known as *zarzamora*, is one of the most important weedy shrubs in Chile and in the 1970s it occupied approximately

5 million hectares. In 1974, the Universidad Austral de Chile conducted studies to introduce the fungus *Phragmidium violaceum* (Schultz) Winter. It was confirmed that this phytopathogen has the advantage of being a specific agent for this weed, without being a major threat to other species (Oehrens and González, 1974), and was then used for control of the weed.

*Biological control of diseases*

Biocontrol of diseases began in Chile with the evaluation and subsequent commercialization of a strain of *Agrobacterium radiobacter* (Beijerinck and van Delden) to inhibit the development of *Agrobacterium tumefaciens* Smith & Townsend in orchards. This product is registered and commercialized under the name Biobacter 84 G. The Universidad Austral de Chile conducted several studies to evaluate disease-controlling bacteria in potato (*Solanum tuberosum* L.). Isolates with antagonistic activity on potato bacterial wilt *Pseudomonas solanacearum* (Smith) (now *Ralstonia solanacearum*) were evaluated and those with high levels of antagonism against the pathogen were selected (Ciampi-Panno *et al*., 1987). Among the bacteria that exhibited high antagonistic potential were some strains of *Pseudomonas fluorescens* (Migula) and an application method was later evaluated. The antagonistic strain FC8, associated with a modification, was more efficient in controlling *P. solanacearum* than its direct application on the tubers. The same university began research in the 1990s to develop formulations in capsules of *P. fluorescens* and *Bacillus subtilis* (Ehrenberg) to control *R. solanacearum*. These formulations were positively evaluated, because they did not influence the antagonistic ability of the strains of the microbial control agent.

A first course on biocontrol of diseases using microbial agents was held in the 1990s. Researchers from the Brazilian Agricultural Research Corporation (Embrapa) and the Department of Plant Health of the Faculty of Agricultural Sciences of the Universidad de Chile taught the course. This stimulated studies on microbial control agents. The research focused on control of diseases caused by *Botrytis*, *Rhizoctonia, Fusarium*, *Erwinia* and *Phytophthora*, among others. Studies with mycopathogenic fungi also began and the antagonistic ability of native *Trichoderma harzianum* (Rifa) strains against *Botrytis cinerea* Pers. in apple trees was evaluated, but they obtained only partial control. Also, the evaluation of solid and liquid fermentation of *Trichoderma* mass-production technologies started.

## 7.3 Current Situation of Biological Control in Chile

### 7.3.1 Introduction

The demand for biocontrol agents has increased significantly recently, which is partly due to the greater interest of agricultural companies and producers in complying with national and international regulations for pesticide use, as well as the need to incorporate these more sustainable technologies in their production systems. This situation has aroused interest among technology-based companies, universities, institutes and research centers in conducting research, technology transfer and commercialization of biocontrol agents.

In 2010, Chile signed the Accession Agreement to the Organization for Economic Cooperation and Development (OECD). By becoming a member, Chile acquired commitments associated with the normalization of agrochemical use and the assurance of a high level of protection for human, animal and environmental health, so that risk is reduced as the sale and use of pesticides decreases. The OECD reported in 2005 that Chile was among the countries with the highest values of agrochemical use worldwide with 0.46 t $km^{-2}$ compared with the mean of 0.21 t $km^{-2}$ in other member countries. The task was not easy and pesticide reduction goals were set for the end of 2014. Unfortunately, these goals were not met, because the consumption of chemical pesticides has increased in the past few years.

Pesticides intended for use in agricultural or forestry, whether they are imported, produced, commercialized or used in Chile, must be previously authorized by SAG. The 2017 SAG list of authorized pesticides has 1215 registered products. Less than 7% are classified in the 'natural substance – biological pesticide – pheromone' category, which includes 37 products for disease control, 22 for insect control and no products for weed control.

According to producers and importers, key factors that discourage registration of biocontrol agents in Chile are high cost, lengthy processing times and the large number of documents to submit. This situation has led companies to prefer registering the biopesticides under a series of names that allow them to avoid registration as pesticides, such as growth stimulants, resistance inducers, plant defence enhancers and biodiversity enhancers. Given the limited number of biocontrol agents registered by SAG, farmers have opted to use those agents that appear on the lists of products for organic production of certifying companies. Two companies in Chile, Ecocert Chile S.A and BioAudita-Eco Garantía (BioAudita), provide services to control, guarantee and certify organic products according to the regulations and standards in force in the country and in the main export markets. They also maintain lists of products for use in organic production systems. Meanwhile, SAG (2017) has a register of 56 biocontrol agents authorized for organic agriculture, but less than 4% of these are on the authorized pesticide list.

Important national companies providing biocontrol agents are Bionativa, Biogram, Biomycota, Biocaf, Biobichos, Biofuture, ControlBest and Xilema, among others. Other companies are spin-offs of universities, such as the Biopacific Company that currently relies on foreign capital to produce and commercialize biological agents. Also, international companies are active on the Chilean market (Koppert, Biobee). Still other companies, such as Natural Chile and Controlbest, have focused on providing all-inclusive services associated with biocontrol, such as pest monitoring and IPM. Many of the above-mentioned companies voluntarily participate in the National Biosupply Network (www.bioinsumos.cl), a technical entity created in 2014 by the Association of Chilean Producers of Biological Control Agents.

Although a lot of work has been done and is ongoing in the field of biocontrol in Chile, there are some issues that still need to be addressed. These concern quality control of commercialized biocontrol agents products, development of effective formulations enhancing the effect of agents, and implementation of technology transfer models allowing knowledge-generating entities, such as universities and research centres, to reach the market with their research developments.

INIA has developed an interesting approach in response to the national demand for microbial control agents. The model starts with the collection, identification and characterization of microorganisms that make up the Chilean Collection of Microbial Genetic Resources (CChRGM). This collection includes 1,857 accessions, of which 1,780 belong to the public collection (Table 7.3) and 77 to the private collection. From the CChRGM, 60% have potential for use as biocontrol agents, especially the entomopathogenic fungi (EPF) with 1,083 accessions, mycopathogenic fungi with 178 accessions and nematophagous fungi with 51 accessions. Furthermore, there is an important working collection of more than 100 EPF accessions. The CChRGM is conserved in the Microbial Genetic Resources Bank (Banco de Recursos Genéticos Microbianos, BRGM), the only public microbial bank in the country and the only South American bank recognized as an International Depositary Authority (IDA) under the Budapest Treaty, which allows it to receive microorganism deposits involved with patents (WIPO, 2012). Henceforth, the Biological Control Technological Center (CTCB) is responsible for assessing the germplasm conserved in the collections. This

**Table 7.3.** Microorganism collection of INIA's Microbial Genetic Resources Bank (BRGM).

| Type of germplasm | Most common genera | Number of accessions |
|---|---|---|
| Bacteria | *Bacillus*, *Pseudomonas*, *Streptomyces* | 29 |
| Entomopathogenic fungi | *Beauveria*, *Lecanicillium*, *Metarhizium*, *Paecilomyces* | 1,083 |
| Phytopathogenic fungi | *Botrytis*, *Colletotrichum*, *Fusarium*, *Phytophthora*, *Rhizoctonia*, *Venturia* | 414 |
| Mycopathogenic fungi | *Clonostachys*, *Fusarium*, *Trichoderma* | 178 |
| Nematophagous fungi | *Arthrobotrys*, *Mortierella*, *Pochonia* | 51 |

centre began operating in 2007 with the aim of strengthening the development of technologies to mass produce pest, disease and weed control agents and contribute to the integrated management of cleaner and more sustainable national agriculture and forestry. It has six lines of research and it had managed 17 projects by 2017 for an amount greater than US$6 million.

### 7.3.2 Use of predators and parasitoids

New parasitoids have recently been introduced, such as *Trioxys pallidus* (Haliday) from Iran to control *Chromaphis juglandicola* (Kaltenbach), an aphid affecting the walnut tree (*Juglans regia* L.). This parasitoid showed a high dispersion capacity and good level of aphid population reduction in walnuts three seasons after their release.

New natural enemies were also imported for an 'old pest', the codling moth *Cydia pomonella* L. in apples, because control was insufficient. However, release of the parasitoid *Ascogaster quadridentata* (Wesmael) did not result in improved control, but researchers from the Pontificia Universidad Católica de Chile identified a new parasitoid of codling moth larvae, *Goniozus legneri* (Gordh), which showed 50% larval parasitism in the laboratory (Zaviezo *et al*., 2007).

In addition to the above-mentioned studies, companies supplying biocontrol agents are evaluating application methodologies under field conditions. Xilema Company, for example, studied a method to release *Eriopis chilensis* (Hofmann) against *Eriosoma lanigerum* Hausmann with the objective to incorporate it into IPM for apple. Their studies demonstrated that releasing 100 and 150 adults of *E. chilensis* per 0.5 ha is effective to reduce the pest.

In the past few years, attention has been centred on a pest that causes important damage in Chile's fruit sector: the grapevine moth *Lobesia botrana* (Denis and Schiffermüller). It was first detected in the central zone of the country. SAG declared the pest under mandatory control in 2014 and 2016 (SAG, 2016). This has motivated the interest of various research groups to study biocontrol alternatives for this important pest. Currently, INIA is evaluating the use of different species of the egg parasitoid *Trichogramma*. They will be used as alternatives for *G. legneri* larval parasitoids and predators of the genus *Chrysoperla*.

Pest control programmes in the forestry sector, developed jointly by public institutions and the private sector, resulted in controlling six pests by using predators and parasitoids. Two pests have been targeted in the implementation of new control programmes. One project concerns control of the woodwasp *Sirex noctilio* (Fabricius), which led SAG to declare mandatory control and the execution of a joint control programme with Argentina. The parasitoids *Megarhyssa nortoni* (Cresson) and *Ibalia leucospoides* (Hochenwarth) are being used to control the woodwasp (Beèche *et al*., 2012). Another parasitoid, *Anaphes nitens* (Girault), is used to control the eucalyptus weevil *Gonipterus platensis* (Marelli) (Valente *et al*., 2017).

### 7.3.3 Use of microbial agents to control pests and diseases

During the past decade, the use of microbial agents as an augmentative biocontrol strategy for pest and disease control has significantly increased. Different species of fungi, nematodes and bacteria that affect pests have been identified in Chile. Nowadays, several of the microorganisms that have successfully controlled pests are being mass produced and commercialized in Chile. Below we summarize the recent research efforts.

#### *Entomopathogenic fungi*

INIA is still the major institute for EPF studies. Evaluation of native strains of *B. bassiana* and *M. anisopliae* to control *Tuta absoluta* (Meyrick) eggs and larvae have recently been prioritized. *Metarhizium* spp. exhibit high control levels, with 80% egg mortality and 90% larval mortality, whereas *B. bassiana* causes 60% egg mortality and 50% larval mortality. Native strains of *M. anisopliae* were also evaluated against the black vine weevil *O. sulcatus* under laboratory conditions and high percentages of larval mortality were obtained, varying as a function of spore concentrations and fungal strain (Gerding *et al*., 2000). Strains of *B. bassiana* and *M. anisopliae* have also been evaluated against the curculionids *A. cervinus* and *O. sulcatus* in raspberry.

Different levels of pathogenicity were found, the most aggressive being *B. bassiana* against *A. cervinus* and *M. anisopliae* against *O. sulcatus*. *Hylamorpha elegans* (Burmeister) is an important cereal and grassland pest in Chile. The Qu-M845 and Qu-M270 strains of *Metarhizium* were evaluated to control it and although both were effective, the second strain showed better results (Rodríguez *et al.*, 2004). Sepúlveda (2015) evaluated the enzymatic and insecticidal activity of six native strains of *Metarhizium* spp. to control *A. superciliosus*, an important minor fruit pest in Chile. All the evaluated strains produce destruxin A and concentrations of 100 mg $l^{-1}$ generate 100% insect mortality.

Mass production of EPF can be based on liquid, solid and biphasic fermentation techniques. Liquid fermentation is habitually used for bacteria. INIA has optimized the mass production of *M. anisopliae* on different substrates (Barra-Bucarei *et al.*, 2016). Also formulation studies were done, in order to maintain the viability and pathogenicity of the strains under field conditions. This is achieved by using inert materials such as solvents, emulsifiers, gelling agents and other additives such as nutrients or stimulants. Since 2007, the CTCB has studied formulations for EPF. First, the use of chitin and its derivatives in formulations was studied; next, granular formulations on the basis of sodium alginate were tested (Gerding-González *et al.*, 2007); and a third study evaluated inverse and encapsulated emulsions.

From 2002 to 2017 INIA sold approximately 26,000 doses (1 dose is needed to treat 1 ha of crop) of different *M. anisopliae* strains. The principal target pests, for which 42% of the doses were sold, were curculionids like *Aegorhinus superciliosus* (Guérin), *A. nodipennis* (Hope) and *Naupactus xanthographus* (Germar), which occur mainly in blueberries.

### *Entomopathogenic nematodes*

Research on EPNs has increased lately, stimulated by the existence of germplasm adapted to the wide variety of climates and soil types in Chile. INIA has conducted assays with strains of *Steinernema australe* Edgington, Buddie, Tymo, Hunt, Nguyen, France, Merino & Moore and *S. unicornum* Egington, Buddie Tymo, France, Merino & Hunt to control *A. nodipennis* (Maldonado *et al.*, 2012), which is a native insect that attacks blueberries at the neck, crown and roots, and *Dalaca pallens* (Blanchard), which causes important grassland losses. These insects spend the greater part of their life in the soil, which complicates their control. INIA is also evaluating strains of the native entomopathogenic nematodes *S. australe*, *S. unicornum* and *S. feltiae* (Fiiipjev) Wouts, Mracek, Gerdin & Bedding to overwintering pupae of *L. botrana*, and several strains are able to cause 40–50% pupal mortality. Use of EPNs would allow lengthening of the control period of this quarantine pest. Further, EPNs have been evaluated to control forest pests. Successful inoculations were made by SAG in 2013 in forest plantations with baits containing the nematode *Deladenus siricidicola* (Bedding), aimed at reducing *S. noctilio* populations and preventing their dispersion.

Other work with EPNs concerns mass production technologies in *in vivo* systems (*Galleria mellonella* Linnaeus larvae). Since 2008, EPNs produced with INIA technology have been applied on 200 ha per year to control *A. superciliosus* and *A. nodipennis* in blueberries. Currently the CTCB is developing *in vitro* production protocols for EPNs by combining different liquid media and is evaluating the productivity of the nematode *S. unicornum* strain QU-N85 and its symbiotic bacteria. *In vitro* production appeared not to affect the parasitic and pathogenic ability of the nematode.

### *Bacteria for control of insects, nematodes and diseases*

Several genera of bacteria have been evaluated to control insects. Native *B. thuringiensis* strains demonstrated high toxicity in *T. absoluta* larvae (Niedmann and Meza-Basso, 2006), *Plutella xylostella* (Linnaeus) and *Agrotis* spp. Commercial formulations of *B. thuringiensis* (Dipel, XenTari, and Turilav) also caused high percentages of *T. absoluta* larval mortality (Ramírez *et al.*, 2010).

Phytoparasitic nematodes are a major problem in many crops. Chemical control and resistant cultivars of fruit are used, but results are not always satisfactory. Rhizobacteria of the genera *Bacillus*, *Brevibacterium*, *Oerskovia* and *Pseudomonas* have been used to control *Globodera rostochiensis* (Wollenweber), a phytoparasitic nematode in potatoes, with *Oerskovia turbata* (Erikson) as the most effective species. Clay and liquid formulations of *Bacillus* and *Pseudomonas* native strains have also been evaluated against nematodes of the genera *Xiphinema* and *Meloidogyne* that affect grapevine.

#### *Fungi and bacteria for control of diseases*

One of the most studied agents to control diseases in Chile is the fungus *Trichoderma* against the following pathogens: *Alternaria alternata* (Keissl) (Roco and Pérez, 2001), *B. cinerea* (Lolas *et al*., 2004), *Cladosporium echinulatum* (Berkeley) (Sandoval *et al*., 2009), *Pyrenochaeta lycopersici* (Schneider and Gerlach) (Besoain *et al*., 2007; Sánchez-Tellez *et al*., 2013) and *Rhizoctonia solani* (Kühn) (Montealegre *et al*., 2010).

In forests, one of the most important pathogens is *B. cinerea*, causing serious damage in nurseries. Seventy-one strains of the antagonistic fungi of *B. cinerea* were evaluated for pathogen colonization and sporulation in *in vitro* assays using leaf discs of *Eucalyptus globulus* Labill. (Molina *et al*., 2006). Strains of the genera *Trichoderma*, *Clonostachys*, *Pencillium* and *Cladosporium* significantly reduced pathogen colonization and sporulation. In addition, strains of *Trichoderma* and *Clonostachys* were evaluated under nursery conditions and the latter achieved a better level of effectiveness against *Botrytis* in *E. globulus* (Zaldúa and Sanfuentes, 2011). Strains of these same genera were evaluated against *Fusarium circinatum* (Nirenberg and O'Donnell) and the *Clonostachys* UDC-222 strain significantly increased seedling survival of *Pinus radiata*. Currently, the biggest forest nurseries of Chile use *Trichoderma* for *B. cinerea* and *F. circinatum* control.

González (2001) evaluated the ability of *Bacillus lentimorbus* Dutky strains to inhibit *Fusarium solani* (Dutky) growth. The bacterium was able to inhibit pathogen growth between 27% and 77%, depending on the method used. Evaluated *Bacillus* spp. strains showed good results against *Erwinia carotovora* (Smith) (Toledo and Sandoval, 2004) and *Phytophthora infestans* (Mont.) de Bary (Rojas and Sandoval, 2009). Several products now found on the market for disease control are based on *Trichoderma* and *Bacillus* and some of them are already on the SAG list of authorized pesticides, while others are in the registration process. It is estimated that annually over 5,000 ha are using products based on microorganisms for control of plant diseases, mainly in orchards and vegetables.

#### *Areas under biological control in Chile*

Currently, according to INIA information (L. Devotto, Quilamapu, Chile, 2019, personal communication), 100% (225,042 ha) of the wheat area and 99% (1,434,085 ha) of the pine area are under classical biocontrol. Although it is complicated to obtain a complete picture of areas under biocontrol, an overview of data is given in Table 7.4. It is stressed that the estimated areas under classical biocontrol (7,726,465 ha) and augmentative biocontrol (62,197 ha) are underestimates.

## 7.4 New Developments of Biological Control in Chile

Until a few years ago, biocontrol of pests in Chile consisted almost exclusively of classical biocontrol. This approach will be continued in the future in spite of increasing restrictions that exist in all countries with respect to Access and Benefit regulations as formulated in the Nagoya protocol (Mason *et al*., 2018).

The Centro de Entomología Aplicada Ltda. (Applied Entomology Center Ltd) is currently developing a toxic bait for use in agriculture to control the Argentine ant *Linepithema humile* Mayr in fruit orchards. Funding is provided by the Foundation for Agricultural Innovation (Fundación para la Innovación Agraria) (FIA). This ant is a limiting factor in the use of natural enemies, because it establishes a mutual relationship with sap-sucking and honeydew-secreting pests from whom it obtains its main food and repels its biocontrol agents.

The Bionativa Company, known in Chile for the production of biocontrol agents for diseases, is developing a hybrid formulation with FIA funding that includes natural extracts and microorganisms to control fruit and vegetable powdery mildew. The objective is to incorporate these formulations when there are no alternatives, or existing alternatives exhibit operational limitations and/or residues for its use. Favourable application times are at post-harvest, sprouting,

**Table 7.4.** Major biological control programmes in Chile.

| Natural enemy/ antagonist | Pest and crop | Type of biocontrol[a] | Area (ha) under biocontrol |
|---|---|---|---|
| *Metaphycus helvolus* | Olive black scale in olives | CBC | 21,904[b] |
| Parasitoids and predators | Scales in citrus, avocado and other fruit | CBC | ~20,000[b] |
| *Cryptolaemus* spp., *Leptomastix* spp. | White mealybugs in citrus, other fruit, alfalfa, potato, olives | CBC | ~130,000[b] |
| *Aphelinus mali* | *Eriosoma lanigerum* aphids in apple | CBC | 37,000 |
| *Trichogramma* spp. | *Cydia pomonella, Rhyacionia buoliana, Tuta absoluta,* and *Lobesia botrana* in forestry, fruit and vegetables | ABC | 2,500 |
| *Metarhizium anisopliae* | *Aegorhinus* spp., *Naupactus xanthographus*, *Asynonychus cervinus, Dalaca pallen, Otiorhynchus sulcatus,* and other curculionids | ABC | 4,372 |
| *Chrysolina* spp. | *Hypericum perforatum* weed, in pastures and nature | CBC | ?, but large |
| Parasitoids and predators | Wheat aphids in wheat | CBC | 225,042 |
| *Orgilus obscurator* | *Rhyacionia buoliana* European pine shoot moth in pine forests | CBC | 1,434,085 |
| *Psyllaephagus pilosus* | *Ctenarytaina eucalypti* eucalyptus psyllid in eucalyptus forests | CBC | 825,000 |
| *Agonopterix ulicetella* | *Ulex europaeus* gorse weed in agriculture and forestry | CBC | ?, but large |
| *Phragmidium violaceum* | *Rubus ulmifolius* weedy shrub in agriculture and nature | CBC | 5,000,000 |
| *Agrobacterium radiobacter* | *Agrobacterium tumefacien* in fruit orchards and berries | ABC | 6,432 |
| *Trichoderma* spp | *Botrytis cinerea* in fruit and vegetables | ABC | 21,162 |
| *Chrysoperla* spp. | Aphids in agriculture | ABC | 100 |
| *Tupiocoris cucurbitaceus* | *Tuta absoluta* and *Trialeurodes vaporariorum* in vegetables | ABC | 20 |
| *Trioxys pallidus* | *Chromaphis juglandicola* aphids in walnut orchards | CBC | 33,434[b] |
| *Megarhyssa nortoni, Ibalia leucospoide* | *Sirex noctilio* woodwasps in pine forests | CBC | 1,419,744 |
| *Anaphes nitens* | *Gonipterus platens*, eucalyptus weevil in eucalyptus plantations | CBC | Part of 825,000 |
| *Bacillus* spp. | *Pseudomonas, Xanthomonas* and *Clavibacter* in fruit, grape and vegetables | ABC | 24,657 |
| Microbials various | Soil diseases in fruit, grape and vegetables | ABC | 30,548 |
| Entomopathogenic nematodes | *Aegorhinus* spp. and *Naupactus xanthographus* | ABC | 580 |

[a]Types of biocontrol: CBC = classical biological control, ABC = augmentative biological control
[b]Area of crop harvested in 2017 according to FAO (http://www.fao.org/faostat/en/#data/qc)

and close to harvest. As for formulations, INIA is developing two initiatives to prepare encapsulated and microencapsulated entomopathogenic fungi and nematodes to increase the field performance of these agents.

With FIA funding, INIA and the Natural Chile Company are executing an initiative to increase the efficiency and sustainability of pest management in agriculture through intensive monitoring and release of natural enemies by unmanned aerial vehicles (UAVs, i.e. drones). The project aims to use pest monitoring field data to prepare flight plans for the UAV, so that releases will spread the necessary amount of natural enemies at specific locations related to the degree of infestation. Another approach is biocontrol and education. Currently, those interested in these technologies have no access to courses at high school, college or university. Almost all the Chilean biocontrol companies were spin-offs from INIA, through former staff, students and/or short-term trainees. To improve this situation, an alliance between INIA and three secondary-level schools was established to offer theoretical and hands-on education on the production and use of biocontrol agents to students who will become agricultural technicians after their training.

The potential of native rhizobacteria to control phytoparasitic nematodes is being evaluated by collaboration between the companies Biorend and Biogram, and the Universidad de Chile along with funding from the Scientific and Technological Development Support Fund (Fondo de Desarrollo Científico y Tecnológico) (FONDEF). In addition, the development of a commercial formulation which is adapted to the production conditions of the central zone of Chile is being pursued.

The Universidad de Concepción, the Universidad de Talca, the Center for Advanced Studies in Arid Zones (Centro de Estudios Avanzados en Zonas Árida) (CEAZA) and INIA study endophytic microorganisms in native species, determine the endophytic colonization ability, persistence and localization of the microorganisms inside plants, and their effect as pest and disease agents, in the following projects:

- CEAZA works with an endophyte of a *Lecanicillium lecanii* (Zimm.) Zare & Gams strain isolated from native plants. Assays demonstrated that the fungus shows activity as a pathogen of adult *Bemisia tabaci* (Gennadius) and *Macrosiphum euphorbiae* (Thomas) and as a mycopathogen against the genera *Rhizoctonia*, *Sclerotinia*, *Botrytis* and *Mucor.*
- INIA established the first collection of endophytic microorganisms isolated in different regions of the country. This collection consists of more than 100 accessions of fungi and bacteria, some of which are being identified to be included in the CChRGM. Particularly the endophytic actinobacteria of native *S. tuberosum* lines to control bacterial diseases and promote potato growth are of interest. It has been possible to isolate endophytic actinobacteria of native potatoes and evaluate them for their antagonistic ability against *Pectobacterium carotovorum* (Jones) Waldee subsp. *carotovorum* and *Pectobacterium atrosepticum* (Van Hall), which cause soft rot and black foot rot in potatoes.
- Another area of research at INIA concerns the endophytic colonization ability exhibited by fungi of the genera *Beauveria* and *Metarhizium* and their potential use for pest and disease control. The colonization ability of native strains of nematophagous fungi of the genera *Beauveria*, *Trichoderma*, *Paecilomyces*, *Clonostachys*, *Fusarium* and *Metarhizium* has also been studied. Studies have been promising, because all the evaluated strains, except *Beauveria*, exhibited some degree of endophytic colonization in tomato seedling, thus implying that it might be an alternative for control of phytoparasitic nematodes of this species.

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Please see the supplementary file "Addenda and Corrections" for names of natural enemies introduced into Chile for control of forest pests.