Control of the soft brown scale, *Coccus hesperidum* L., in greenhouses of botanical gardens

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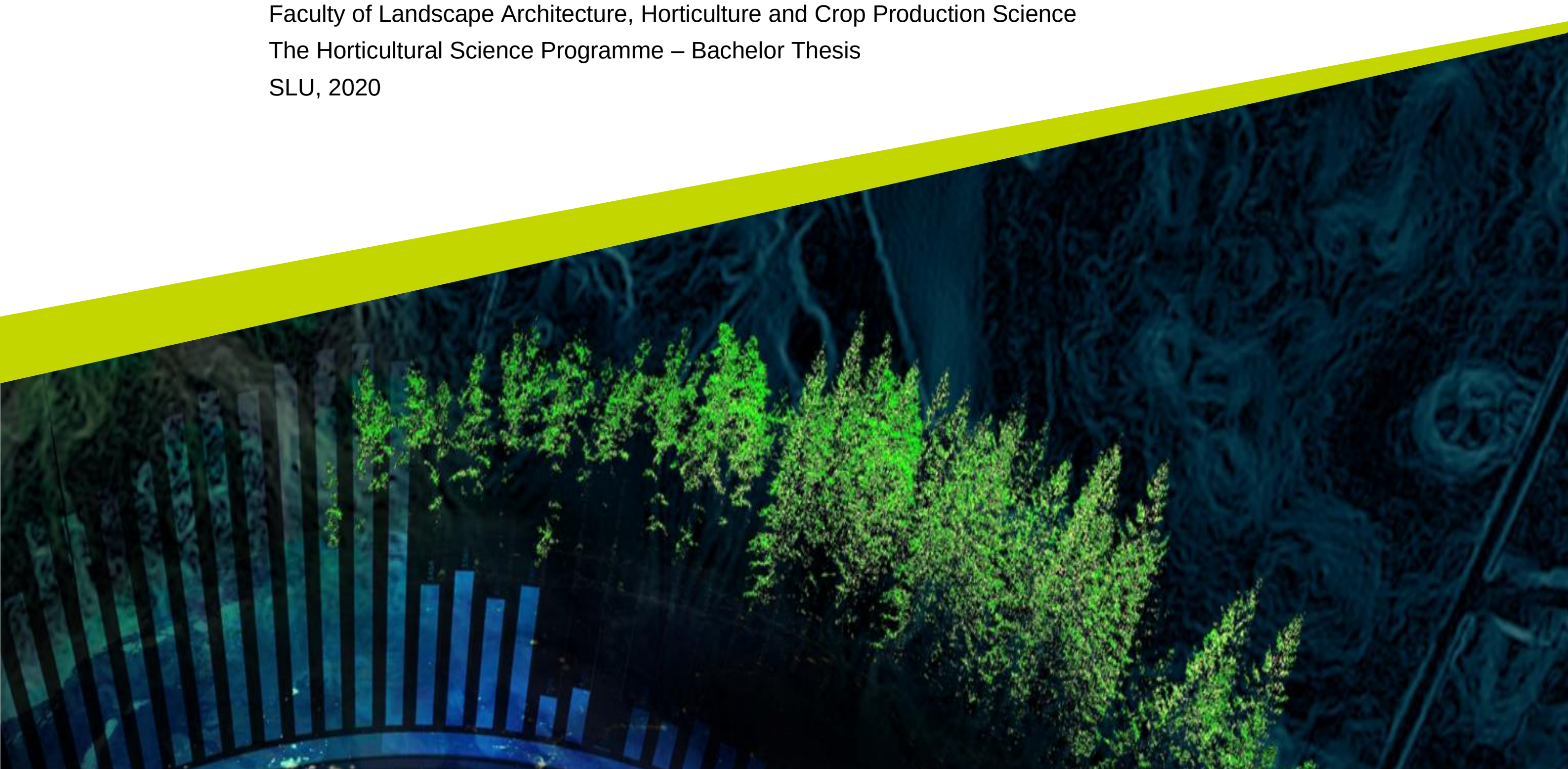

Control of the soft brown scale, *Coccus hesperidum* L., in greenhouses of botanical gardens

Kontroll av orangefärgad vaxsköldlus, *Coccus hesperidum* L., i växthus i botaniska trädgårdar

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Abstract

In this bachelor thesis, the control methods for the soft brown scale, *Coccus hesperidum* L. in greenhouses of botanical gardens, were discussed based on the knowledge gained from a questionnaire answered by gardeners from botanical gardens.

C. hesperidum is a common pest in greenhouses. However aggressive ants make it difficult for the natural enemies, released as biological control, to parasitize and predate the pest. The greenhouses of botanical gardens are artificially made environments in semi-closed ecosystem, and the dynamics and the environment in the greenhouses can easily be disturbed. The botanical gardens have rules and normal procedures, which the gardeners have to respect. The preferred method of control is biological control, but in exceptional cases chemicals could be used.

This study aimed to examine which methods are suitable in the greenhouses and where further research is needed.

I found that *C. hesperidum* had many different methods of control. Biological control does not always work as intended because it affects many different trophic levels that have to be accounted for. Chemicals can be necessary, if the natural enemies of *C. hesperidum* do not work as expected. Pheromones are used for bigger crops, and further research for non-chemical treatment for smaller use is needed. Spread and arrival of new pests can often be avoided.

Keywords: *Coccus hesperidum*, soft brown scale, plant protection, botanical garden, greenhouse, biological control.

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Caroline Bayer Frøhling

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1 Introduction

Greenhouses in botanical gardens can suffer pest attack from different sources; the pests can come in through the window or arrive along with new plant material (Anom., 2020b). Once the pests are inside the greenhouses, they cannot be exterminated, and further spread should be avoided. The greenhouses of botanical gardens are artificially made environments in semi-closed ecosystems. New pests can easily disturb the ecosystem on different trophic levels. The botanical gardens have rules and normal procedures, which the gardeners have to follow. It is important to use the right methods of control, when discovering a new pest attack. The botanical gardens use biological control, except in exceptional cases like attacks on especially important or vulnerable plants, or where there are no possibilities for biological control.

The soft brown scale, *Coccus hesperidum*, is a common pest in greenhouses and can easily be controlled using its natural enemies, but aggressive ants can disturb this treatment. Usually, gardeners use *Microterys flavus* and *Coccophagus lycimnia* to manage attacks of *C. hesperidum*, but ants can protect *C. hesperidum* against the natural enemies (Kapranas et al, 2007; Anom., 2020a). *Cryptolaemus montrouzieri* can be used as biological control against *C. hesperidum*, but only in the absence of mealybug, since *C. montrouzieri* lay their eggs in the egg-collections of mealybugs (Kullin, 2008).

C. hesperidum can be treated with chemicals like chlorpyrifos, Buprofezin and the microbial derivative spinosad (Shivanna et al, 2011). These pesticides also have negative effects on non-target insects such as natural enemies of *C. hesperidum* and other insects (Suma et al, 2009). This could disrupt the dynamics of the artificially made ecosystems in the greenhouses and also the control of other pests.

Insecticidal baits could be a possible and effective way of treating *C. hesperidum* with a low dose of insecticides. Sunamura et al (2011) state that the insecticidal baits are not effective in themselves, but in combination with a synthetic trail pheromone effects have been shown. According to Warner & Scheffrahn (2004), non-target insects will not be affected by the baits, while Suckling et al (2010) claim the opposite. Further research on the efficacy and specificity of the bait will be needed to get a clearer picture.

Trail pheromones of ants are a debated area with no clear picture; Sunamura et al (2011) debate they are not effective by themselves, while Suckling et al (2010) claim that pheromones do work. ‘Rope’-type pheromone dispensers in combination with baits have been shown effective (Sunamura et al, 2011).

C. hesperidum can be removed mechanically by the gardeners (Anom., 2020b), but there is a risk of damage to the plant, and it can be time consuming on a highly infested plant.

The research on how to control *C. hesperidum* is vast, but lacks findings of treatments in the greenhouses of botanical gardens, which have rules and normal procedures to follow. *C. hesperidum* needs to be controlled in greenhouses of botanical gardens by means of ways to affect the artificially made ecosystem in the least negative way, and taking into consideration the rules and normal procedures of the botanical gardens.

1.1 Aims

The aim of this bachelor thesis is to examine how to control *Coccus hesperidum* in greenhouses of botanical gardens, through knowledge about the lifecycle and spread of the species, as well as its predators and other species affecting the predator/prey relationship between them. By obtaining knowledge of plant protection in greenhouses in botanical gardens, it is possible to take into account the rules and normal procedures of the botanical gardens, when controlling *C. hesperidum*.

The following questions were specified:

- How can *C. hesperidum* be controlled in greenhouses of botanical gardens by means that affect the artificially made ecosystem in the least negative way?
- How can *C. hesperidum* be controlled in greenhouses of botanical gardens in the most suitable way, when taking into consideration the rules and normal procedures of the botanical gardens?

This bachelor thesis does not include economic aspects and legislation.

2. Materials and Methods

This bachelor thesis was carried on as a literature study based on, and in combination with, a questionnaire, personal contact and follow-up e-mails.

I created the questionnaire ‘Plant protection with a focus on arthropods in greenhouses in botanical gardens’ based on my interest and knowledge of plant protection, arthropods, and botanical gardens. It was designed to get a general perspective on plant protection in regards to arthropods in botanical gardens, as well as getting specific examples of existing problems. It consisted of 15 questions, of which 12 concerned plant protection with a focus on arthropods in greenhouses in botanical gardens. The persons responsible for the plant protection in greenhouses of the respective botanical gardens answered the questionnaires. See Appendix 1 for the complete list of questionnaire items.

The questionnaire was sent as a link in an e-mail to four different botanical gardens in Denmark and Sweden; the botanical gardens of Copenhagen (DK), Aarhus (DK), Gothenburg (SE), and Lund (SE). The selection of botanical gardens was based on my prior knowledge of their existence. Only the botanical gardens of Aarhus and Gothenburg answered and follow-up e-mails were exchanged with Aarhus (Lang, 2020, pers. comm.). Gothenburg did not reply to the follow-up questions.

A personal meeting about plant protection with a gardener in the botanical garden of Copenhagen (Anom., 2020a) took place, and follow-up e-mails were exchanged (Anom. 2020b).

Based on the questionnaire, meeting and follow-ups, I chose to focus on the control of *C. hesperidum* in greenhouses of botanical gardens. Literature was collected from scientific articles from the databases such as Primo and Google Scholar, from books, and from the webpages of suppliers of products for biological plant protection.

3. Results

3.1 Summary of questionnaires and contacts with botanical gardens

All three botanical gardens use biological control, and Gothenburg and Copenhagen only use chemicals in exceptional cases. Such cases can be pest attacks on especially important or vulnerable plants, which cannot withstand too much damage, or where there are no possibilities for biological control (Anom. 2020b).

Both Aarhus and Copenhagen specify using insect soaps and oils, and Copenhagen uses agro technological solutions like sprinkling, rinsing with water, mechanical removal and pruning. Gothenburg mentions sanitation and emphasizes prevention as a plant protection method, and states that discovery, identification and actions from the gardener are important.

All three botanical gardens have release schedules for beneficials, i.e. predators or parasitoids of pests, which are continuously updated.

All botanical gardens specify the new plant material as a source of new pests and Copenhagen has noted that pests also come in through the windows.

All three botanical gardens state, that they get their new plant material from other botanical gardens, but they do not specify which botanical gardens. Aarhus gets new plant material from private individuals and mentions getting plant material from other countries, furthermore Copenhagen get their new plant material from plant distributers as well (Anom. 2020b).

They all specify having problems with scale insects. A gardener in the botanical garden of Copenhagen (Anom., 2020a) showed me an example of a plant with an infestation of possibly *C. hesperidum*. He mentioned that it started thriving after the arrival of a novel ant species, and that the natural enemies (*Microterys flavus* and *Coccophagus lycimnia*), which are normally used as biological control, were not working anymore. He also stated that the natural enemy of mealybugs, *Cryptolaemus montrouzieri*, had started thriving. The gardener in the botanical garden of Copenhagen (Anom., 2020b) specifies *Technomyrmex albipes* or *T. vitiensis* as possibly being the novel ant species. Gothenburg lists *Tapinoma sessile* as problematic, because they take care of and protect scale insects, mealybugs and aphids against certain natural enemies.

Lang (2020, pers. comm.) points out that half of the time the praxis does not follow the theory. They have many examples of natural enemies not working as they were supposed to. Lang (2020, pers. comm.) also mentioned that they never quite manage to exterminate the pests, as they cannot reset the greenhouses because they have plants, heat and light in the greenhouses all year round.

3.2 Greenhouses in botanical gardens as ecosystems

Ecology describes the interactions of living organisms and their environment (Loreau, 2009). An ecosystem is the system of abiotic and biotic factors and their interactions in a location. Everything from sunlight to soil is accounted for when describing the mechanisms of an ecosystem, but everything cannot be controlled in a greenhouse, and the different organisms affect each other.

The greenhouses in botanical gardens are artificially made, semi-closed, controlled environments; they have systems in place for controlling temperature, sun exposure, humidity, irrigation. Gardeners control what goes in and out of the greenhouses, such as nutrition, soil, decomposing plant material and new plant material, and they fight pests and irrigate. There are some factors that gardeners cannot control entirely, like diseases, weeds and pests from outside sources. The botanical gardens of Aarhus, Copenhagen and Gothenburg specify the source of new pests as new plant material, and as mentioned before Copenhagen has noted that pests also come in through the window. They get their new plant material from other botanical gardens, and Aarhus gets new plant material from private individuals and mentions getting plant material from other countries and Copenhagen get their new plant material from plant distributers as well (Anom., 2020b). Even though they are controlled ecosystems, Lang (2020, pers. comm.) states that they can never manage to exterminate the pests because they cannot "reset" the greenhouses because they have plants, heat and light in the greenhouses all year round.

Botanical gardens are living museums with the purpose of conserving living plant material of many different species, and educate the public about plants, as well as being recreational spots (SNHM, 2020).

3.3 *Coccus hesperidum* L.

The species *Coccus hesperidum* L. (*C. hesperidum*), or the soft brown scale, is a member of the superfamily Coccoidea (Order: Hemiptera), which contains many horticulturally important pests.

3.3.1 Appearance and lifecycle

Throughout this bachelor thesis, focus will be on the females, unless otherwise indicated. According to Krapanas (2012), the females of *C. hesperidum* is an oval-shaped small scale insect, measuring no more than 4-5 mm. Often it is possible to find them in many different sizes because they are multivoltine, which means there are multiple generations per year. Their body is greenish-brown to yellow-brown in color (Sheehan, 1992) and generally flat, as seen in figure 1, but becomes more convex with age (Krapanas, 2012). They are wingless and have piercing mouthparts (Crop Knowledge Master, 1992; Cranston & Gullan, 2014).

They exhibit extraordinary sexual dimorphism, due to the fact that the males and females have very different life styles and life spans. The males are rarely observed because the species display parthenogenesis, where the females reproduce without fertilization (Krapanas, 2012). Males are either wingless or have one pair of forewings with reduced venation, and they look like tiny flies (Crop Knowledge Master, 1992; Cranston & Gullan, 2014). The males live very short lives; they fly around for a few hours or a day or two to find a mate and after that, they die. Since their life span is so short, they do not have to eat and they have reduced mouthparts, therefore the males are not considered as pests (Cranston & Gullan, 2014).

The females have three stages, two nymph stages and one adult stage. It looks similar in all life stages, except from the size and the fact that the nymphs, or "crawlers", move slightly. The third stage (adult stage), is spent sedentarily, while they eat and reproduce. They live for up to four months and have overlapping generations (Crop Knowledge Master 1992).

As mentioned, *C. hesperidum* display parthenogenesis, which is generally rare in both the animal and plant kingdoms, but widespread among the different groups of homoptera (Vrijenhoek & Parker 2009). It involves the females reproducing asexually, without fertilization by sperm from the males. Parthenogenesis sacrifices genetic variation by reproducing clonally, but the energy cost of producing males and risks when finding mates, is avoided (Vrijenhoek & Parker 2009). They claim that species displaying parthenogenesis are more common in northern parts of the world. *C. hesperidum* display both sexual and asexual reproduction, but this could be geographically limited, as males have been observed more frequently in northern Europe than in southern Europe (Krapanas, 2012).

Ovoviviparity is when, after fertilization of the eggs, the eggs are incubated inside the reproductive tract of the female. When the eggs are fully developed, they hatch immediately after being laid or just before being ejected from the females reproductive tract (Cranston & Gullan, 2014). It is the last-mentioned strategy that *C. hesperidum* utilizes. The insects that lay eggs have an egg-laying tube (ovipositor), but it is absent in *C. hesperidum*.

Figure 1. *Coccus hesperidum* flat on leaf surface (by cquintin (CC BY-NC 2.0)).

3.3.2 Damage and horticultural importance

C. hesperidum is polyphagous, which means it can feed on multiple plants. Since it is primarily sedentary, they usually only feed on one plant throughout their lives, but they are able to feed on others. They feed exclusively on living plant sap from the phloem, which transports nutrients from the leaves to the storage organs. As mentioned, they have piercing mouthparts, which they use to penetrate the cells in the plant stem or midrib of the leaf (Cranston & Gullan, 2014). The protein content in the plant sap is low and the sugar content is high, and the *C. hesperidum* has to imbibe plentiful amounts to get sufficient protein. The excess carbohydrate is excreted and left as a sticky film, or honeydew, on the leaves (Kullin, 2008). This can give rise to black sooty mold (see section 3.4) and mutualistic relationships with ants (see section 3.5).

The direct damage caused by *C. hesperidum* is not that apparent, as the cell walls are left intact after the withdrawal of the sap from the phloem, but it can lead to wilting, distortion, or stunting of shoots (Cranston & Gullan, 2014). It is difficult to determine exactly how much it damages the individual plant, but it does drain plant resources, which can cause retarded root growth, fewer leaves and/or less overall biomass accumulation (Cranston & Gullan, 2014). *C. hesperidum* can be difficult spot if they are located only on the branches, but when the leaves are infested, they are clearly visible (Cranston & Gullan, 2014). One of the objectives of botanical gardens is recreation, and a highly infested plant can be aesthetically unpleasing and unappealing.

C. hesperidum is not considered a serious pest, except on ornamental plants and on citrus, mangos, guava, and lychee (Kapranas et al, 2007). It is only when it is having mutualistic relationships with ants, that it achieves pest status and becomes economically damaging (Kapranas et al, 2007).

3.3.3 Origin and spread

C. hesperidum has a cosmopolitan distribution, which means it is distributed across most of the world, and it is often found on citrus throughout the Mediterranean area. As mentioned above, it display parthenogenesis and the observation of the males is more frequent in northern Europe than in southern Europe (Krapanas, 2012). It possibly has Afro-Ethiopian, Oriental and/or Astro-Oriental origin, but this is not for certain.

Several unintended insects accompany global trade of plant material (Cranston & Gullan, 2014). *C. hesperidum* can easily be distributed around the world on ornamental plants, since they stick to the plant, where they have food and shelter. They can spread quite rapidly on a single plant, because they have multiple generations per year (Kullin, 2008). They are mainly sedentary, which means the most frequent method of spread to other plants is by wind (Krapanas, 2012).

3.4 Black sooty mold (*Cladosporium* spp.)

The presence of *C.* hesperidum can give rise to sooty molds, which are caused by a fungus of the order Capnodiales and appear as a matt, black film on the surface of stems and leaves of plants (Agrios, 2005). They are most common in humid, warm weather and can be found on all types of plants because the fungus does not penetrate the cells of the plant and are therefore not dependent on a certain kind of plant nutrition. It is dependent on the sugary fluid from honeydew for the mycelium to grow. In severe cases, the fungal growth can get so abundant that it impairs photosynthesis, which can lead to biomass accumulation loss (Kullin, 2008; Cranston & Gullan, 2014). Because it does not penetrate the cells of the plant, the black layer can be removed by wiping the leaves with moistened cloth (Agrios, 2005).

There are no control measure applied directly against sooty molds, but by eliminating the source of the honeydew, i.e. the honeydew-producing insects, it is possible to control the fungal growth (Agrios, 2005).

3.5 Mutualistic relationship with ants

Ants (Hymenoptera: Formicidae) are tremendously successful and are one of the most prominent groups of arthropods (Ward, 2006). There are approximately 20,000 species and they inhabit all landmasses, except from the poles and high altitude locations. Most species are *eusocial*, which means generations are overlapping and they have division of labor. There is the reproductive labor, and the non-reproductive worker caste, which searches for food and brings it back to the nest. The worker ants consist almost entirely of wingless females and have a communication system consisting of pheromone trails to find their way to food resources and back to the nest, which is unique for ants compared to other insects.

Mutualism is an ecological interaction between two species (partners) that benefits both of them (Bronstein, 2015). The interactions can be classified according to how the different partners benefit from the interaction. It can be *transportation*, which is the movement of partners or their gametes. It can be *protection*, where one of the partners defends the other against biotic factors, or it can be *nutrition*, where one partner provides important nutrition for the other partner. They can be grouped by looking at what the partners exchange in their interaction (Bronstein, 2015).

The benefits can be *direct*, where the contact between the partners are direct or the benefits can be *indirect*, where one of the partners affect a third species (Bronstein, 2015).

Hemipterans have mutualistic relationships with ants; they provide a food resource for the ants, and in return, the ants protect them from predators. Hemipterans produce honeydew, which serves as an important carbohydrate source for the ant, which in return will defend them from their natural enemies (Cranston & Gullan, 2014).

The relationship is a protection-nutrition relationship. The benefits of the nutrition relationship is direct, seeing as the ant get honeydew directly from the scale insect. The benefits from the protection relationship is indirect, since the ants protects the scale insect from another species; the natural enemies of *C. hesperidum*.

Ants can show aggression towards other arthropods, including the enemies of *C. hesperidum*, which leads to a lower predation rate on the *C. hesperidum*. This leads to an increased number of *C. hesperidum*. Ants can even display aggression against other ant species (Cranston & Gullan, 2014).

Buckley & Gullan (1991) found that ants can be more or less effective, when protecting other insects. The aggressiveness of the ant towards natural enemies of the coccoids increases the number of coccoids. Ants are polyphagous, but the different species have different palates and thus have different preferences in sugar composition (Warner & Scheffrahn, 2004).

The gardener in the botanical garden of Copenhagen (Anom., 2020b) identifies *Technomyrmex albipes* or *Technomyrmex vitiensis* as being the possible novel ant species, and Gothenburg lists *Tapinoma sessile* as problematic, because of their mutualistic relationships with scale insects, mealybugs, and lice.

Blatrix et al (2018) claim that greenhouses are often dominated just by one or two species of ants.

3.6 Control

This section examines control of *C. hesperidum* on different trophic levels with different approaches.

3.6.1 Prevention and sanitation

Integrated Pest Management (IPM) is a concept of integrating methods in plant production that reduce the use of chemicals (Pettersson & Åkesson, 2011). Firstly, preventive measures must be taken, like regular cleaning of machinery and equipment (The European Commission, 2020). One of the points of IPM (IPM info, 2020) is to use clean plant material, so that pests, especially scale insects, do not pass to the next crop with infested plant material. Then ecological measure can be taken, like biological control or biorational synthetic volatiles can be used (Stenberg, 2017). As a last resort chemical pesticides can be used.

The botanical garden of Gothenburg also mentions sanitation and emphasizes prevention as a plant protection method. Prevention and sanitation could be an easy way of avoiding pest attacks. Some of the routines already in place could need an update or entirely new routines should be added.

3.6.2 Mechanical removal of the scale insects

Nyttedyr (2020) refer to mechanical removal of the brown soft scale for private users and to be observant of when the eggs hatch. At the young stages, they can be treated with insect soap or finishing spray. Copenhagen uses agro technological solutions such as mechanical removal.

3.6.3 Biological control

There are several host-specific natural enemies of C. hesperidum, which are often used in biological control (see Table 1.). Biological control does not exterminate all pests, but reduce the abundance of the target pests (Gullan & Cranston, 2014), so continuous control is necessary to keep the population down and the damage at a minimum.

Pests	**Natural enemies**
Aonidiella aurantii	*Aphytis melinus*
Coccus hesperidum	*Microterys nietneri* *Coccophagus lycimnia* (*Cryptolaemus montrouzieri*)
Mealybugs	*Cryptolaemus montrouzieri*
Planococcus citri	*Leptomastix dactylopii*
Saissetia coffeae	*Coccophagus lycimnia*
Saissetia oleae	*Coccophagus lycimnia*

Table 1. Some of the pests in the greenhouses and their natural enemies.

Microterys nietneri (Motschulsky) (formerly known as *Microterys flavus* (Howard)) is placed in the family Encyrtidae, which is a family of parasitic wasps. They are yellow-brown in color and the females are about 2 mm in size. They can kill both smaller and larger soft brown scales by feeding on them. They are primary parasitoids, which means they lay their eggs inside of the host, without killing it right away (Kapranas et al, 2007). Depending on the size of the host, the adult lay one to several eggs inside of it. If the host is big, there is room for more eggs. The eggs develop into new parasitoids, which feed on the host. The adults feed on the younger soft brown scales by puncturing them and sucking them empty (Entocare, 2020).

Coccophagus lycimnia (Walker) (Hymenoptera: Aphelinidae) is a small parasitoid wasp on various scale insects. These parasotoids have a cosmopolitan distribution and live in greenhouses in northern Europe (The Bug Guide, 2017). It is commonly used against *Saissetia coffeae* and *Saissetia oleae*, which are in the same family (Coccidae) as *C. hesperidum* (Kullin, 2008), and can be used against *C. hesperidum* with positive effects (Anom., 2020a).

The mealybug ladybird, *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae), is a common predator of mealybugs (Pseudococcidae) and in some cases scales and aphids. The females can grow to the size of 6 mm and the adults have dark-brown elytra, while the head, antennae, pronotum, and legs are orange-brown (Wikipedia, 2020). The larvae are about 14 mm in size; they are whitish and striped with waxy rays protruding from the body, which makes them look like giant mealybugs (Kullin, 2008). Mealybugs have mutualistic relationships with ants too and the appearance of *C. montrouzieri* is possibly a disguise to protect them from ants protecting the mealybugs (Cranston & Gullan, 2014). Mealybugs are oviparous (Pacheco da Silva et al, 2017), which means they lay eggs (Cranston & Gullan, 2014), and the adult *C. montrouzieri* lays its egg in the collections of the eggs of the mealybugs. When *C. montrouzieri* eggs hatch, the larvae eat the mealybug eggs (Kullin, 2008). Both the adult *C. montrouzieri* and the larvae feed on the mealybugs. The adults feed only on the eggs, while the larvae feed on mealybugs in all stages. In absence of mealybugs, *C. montrouzieri* are able to prey on *C. hesperidum* (Kullin, 2008).

For biological control of mealybugs, *C. montrouzieri* can be applied as both adults and larvae, which feed on mealybugs. The adults have the highest effect if they are released, when the concentration of mealybug eggs is high (Borregaard, 2020). This stimulates egg laying and assures that the adult does not fly away. Borregaard (2020) recommends using the larvae in small spots or on solitary plants.

Krapanas (2012) states ant exclusion as a necessity because it enhances parasitism and predation of the *C. hesperidum*.

3.6.4 Pheromone treatment of ants

Pheromones are chemicals used for communication between insects of the same species (Bakthavatsalam, 2016). Ants deposit pheromones to lead other ants on the correct trail to the food source. The pheromone trail works by letting the ant know if it is making an error, when walking to or from the desired destination (Czaczkes et al, 2006). However, it is unclear whether it helps the ants memorize the route. Pheromones do not have an insecticidal effect, which means that the number of ants does not decrease, when using pheromones (Sunamura et al, 2011).

Suckling et al (2010) show that in the presence of large amounts of a specific pheromone, the trailing fire ants became disorientated, and indicate that this may have negative effects on colony size. Suckling et al (2010) further state that this behavior has been demonstrated for two weeks on the Argentine ant using another specific pheromone.

Suckling et al (2007) tested trail disruption in small outdoor plots on the Argentine ant. The pheromone formulation was applied by a rotary spreader, and the ants were counted on bait cards. Ant counts showed a significant reduction in the treated plots over the untreated plots, and ant foraging was significantly reduced for two days.

Cranston & Gullan (2014) claim that trail-marking pheromones are not necessarily species-specific because several species excrete the same chemicals.

Sunamura et al (2011) used 'Rope'-type pheromones dispensers to distribute the specific pheromones, and found it efficient in the combination with baits, but non-efficient alone. These dispensers could be used in greenhouses, but it is possible that an identification of the ant would be necessary.

3.6.5 Chemical treatment

Scale insects have a waxy layer, through which chemicals have difficulty penetrating. Combined with the fact that the adults are sedentary, they are difficult to control with contact insecticides. The young do move and are crawling on the plant. They can be treated with contact insecticides. Systemic insecticides move through the plant in the xylem and phloem and are effective against insects with piercing-sucking mouthparts, such as *C. hesperidum* (Cloyd, 2008).

Shivanna et al (2011) tested six different chemical control agents, including the synthetic chemicals chlorpyrifos, Buprofezin and the microbial derivative spinosad on *C. hesperidum* on the arecanut plant. Chlorpyrifos is an organophosphate insecticide, which is used in agriculture. It works by malfunctioning the nervous center by blocking an enzyme that controls the messages travelling between nerve cells. Chlorpyrifos is harmful to human health (NPIC, 2010). Buprofezin inhibits chitin synthesis and exposure delays the developmental period in the progeny (Ullah et al, 2019). Spinosad also affect the nervous system of insect by contact or ingestion. It affects the muscles and leads to paralysis, and death occurs within 1-2 days (NPIC, 2014). Shivanna et al (2011) found that the chemical control agents had significant effects on *C. hesperidum* over the untreated specimens. The results showed the insecticides to be significantly superior compared to the untreated control, by recording a lower number of scales on the plants treated with the chemicals.

Pesticides can have side effects on non-target insects such as natural enemies. Suma et al (2009) carried out laboratory trials of the side effects of the insecticides chlorpyrifos-methyl, Buprofezin, and spinosad on *Aphytis melinus*, *Coccophagus lycimnia*, and *Leptomastix dactylopii*. All tests done by Suma et al (2009) was done on females by contact with the chemical and they measured the fertility of the females and the sex ratio of the progeny. Just 24 hours after the treatment with chlorpyrifos-methyl and spinosad, 100 % mortality was observed in all tested parasitoids.

The use of chlorpyrifos of Buprofezin has been registered for use against the main arthropod pests of citrus in Spain in 2008 (Ciancio & Mukerji, 2010). Otherwise, the literature on the use of insecticides on *C. hesperidum* is limited and the efficacy of the insecticides in greenhouses needs to be researched further.

Insecticidal baits against ants deliver small amounts of insecticides and are easy to use for the applicator (Warner & Scheffrahn, 2004); the containers are put down in the area of the undesired ants (Ecostyle, 2020). Warner & Scheffrahn (2004) states that baits will not affect non-target ants or insects, unless the composition of the sucrose in the bait is palatable to the ant, and that the bait can be placed in a container, which is specified only to allow in the targeted ants. It was not specified how the container works.

Suckling et al (2010) point out that insecticidal baits are widely used for control, but that they also affect non-target ants and that baits are not appropriate for sensitive ecosystem.

Sunamura et al (2011) state that a combination of synthetic trail pheromones and insecticidal bait may be a more effective way of controlling, by inhibiting a re-infestation by the use of pheromones. Sunamura et al (2011) further state that the two methods separately are not effective and a combination may be a more environmentally friendly method, since the dose of insecticides is low. Sunamura et al (2011) tested three different types of treatment on ant populations: pheromones + baits, only pheromones and only baits (and a control). It showed that the plots treated with both pheromones and baits were the only treatment that maintained ant population at a level lower than or similar to the initial level. It further showed that the number of ants was constantly lowest at the plot compared to the other treatments. In plots only treated with pheromones, there was no evidence of a decline in the ant population. Plots treated with only baits first showed a decline in the number of ants, but the population rapidly recovered or increased after a while.

The greenhouses in the botanical garden of Copenhagen and Gothenburg do not use chemicals unless absolutely necessary. Baits might be suitable, seeing as they use a small dose of insecticides, but opinions on the efficacy and specificity are ambiguous.

4. Discussion

The aims of this study was to examine how to control *Coccus hesperidum* in greenhouses of botanical gardens. This study shows that control of *C. hesperidum* is possible in greenhouses of botanical gardens. There are many options for control and possibility for combinations of a more thorough control, but it is important to consider the rules and normal procedures of the individual botanical gardens. Further research is necessary to give a more detailed picture.

C. hesperidum is a common pest of the greenhouses in botanical garden (Anom., 2020a), and the spread of it is more or less avoidable. Once the pests have entered the greenhouse, they cannot be eliminated completely, because it is not possible to "reset" the greenhouses. Regular cleaning of equipment is necessary to avoid the spread of pests (The European Commission, 2020). The botanical garden of Gothenburg mentions that part of their strategy is to educate their gardeners to discover, identify and take actions, which could decrease the number of attacks of *C. hesperidum* and other pests.

4.1 Effects on the artificially made ecosystems

The ecosystems in greenhouses of botanical gardens are sensitive and the niches can be affected by small changes. The plants are not solitary, but part of a bigger artificially-made ecosystem, and controlling pests can affect other dynamics of the different ecosystems in the greenhouse. Contrary to conventional plant production, every individual plant is important, they have different needs, and different pests are attracted to the individual plants.

Microterys nietneri and *Coccophagus lycimnia* are not effective in the presence of aggressive ants (Anom., 2020a). In order for the two natural enemies to be effective, ant exclusion would be necessary to enhance their chances of parasitism and predation (Krapanas, 2012). The greenhouses never get rid of pests entirely (Cranston & Gullan, 2014), so the ant exclusion would have to be for the period of time it takes for *M. nietneri* and *C. lycimnia* to keep the population down. Ant exclusion could be done by either baits or with a synthetic pheromone trail, but further research is needed on the topic.

Control with *C. montrouzieri* is possible, but only in the absence of mealybugs (Kullin, 2008; Anom., 2020a). Ants show more aggression towards the natural enemies of *C. hesperidum* than towards to *C. montrouzieri*, (Kullin, 2008; Cranston & Gullan, 2014). Mealybugs lay eggs (Pacheco da Silva, 2017), while the *C. hesperidum* is ovoviviparous and gives birth to live nymphs (Cranston & Gullan, 2014). This means that *C. hesperidum* only works as a food source for the adult *C. montrouzieri*, whereas mealybugs works as both food source and a location for laying eggs for the adult *C. montrouzieri*. This also means that the adult female *C. montrouzieri* has to search for other places to lay their eggs, and might stray away from *C. hesperidum* to do so. When finding a suitable spot for egg-laying, *C. montrouzieri* has no reason to return to *C. hesperidum* (unless, there is a lack of food or to find a mate). This is why the adult *C. montrouzieri* only thrives on *C. hesperidum* in the absence of the mealybug. Therefore it is important to control mealybugs, before using adult *C. montrouzieri* directly as biological control against *C. hesperidum*.

As an alternative, it is possible to use the larvae of *C. montrouzieri*, which would stay for a longer time, as they do not need a location for laying eggs yet. They do not have wings, so they will not leave so easily.

Ants can be more or less effective, when defending other insects (Buckley & Gullan, 1991), so it is important to make sure, that more aggressive ant species do not arrive and disturb the biological control in the botanical gardens. Ants can arrive with new plant material as well, and proper inspection is again important to not disturb the dynamics of the ecosystem. It is important to underline that ants do not disappear, the abundance of them is just lowered, so it would be necessary to have a continuous control of them.

Pheromones have no insecticidal effect on ants, and it is unclear where the ants relocate to, when using pheromones. Because the greenhouses in botanical gardens are semi-closed ecosystem, the ants would stay inside. Ants tend other honeydew producing insects, like aphids and mealybugs (Cranston & Gullan, 2014). There is a possibility of just relocating the ants to other parts of the greenhouse. Further research of the relocation of ants in greenhouses is needed. Tests with pheromone treatment in semi-closed ecosystems is needed to give a better picture of how they work.

Chemical insecticides have proven significant effects on *C. hesperidum* (Shivanna, 2011), but also affect other insects, including natural enemies of *C. hesperidum* (Suma et al, 2009). *Aphytis melinus* is a natural enemy of, and used to control the scale insect, *Aonidiella aurantii* (Gonzalez-Zamora & Castillo, 2018), and *Coccophagus lycimnia* can be used against *Saissetia coffeae* and *Saissetia oleae* (Kullin, 2008), which are common in the greenhouse of the botanical garden of Copenhagen (Anom., 2020a). The botanical garden of Gothenburg uses *Leptomastix dactylopii* as a biological control, which is a natural enemy of *Planococcus citri*. The gardener in the botanical garden of Copenhagen (Anom., 2020a) mentioned *P. citri* as a pest in the greenhouse. The mortality rate by use of the chemicals was 100 % on *A. melinus*, *C. lycimnia*, and *L. dactylopii* (Suma et al, 2009).

Gothenburg lists *L. dactylopii* as a biological control used both preventively and regularly when an attack occurs, and it is a commonly used as a natural enemy of *P. citri* (Cloyd & Dickinson, 2006). If insecticidal chemicals are used and *L. dactylopii* gets into contact with it, it would cause problems in other parts of the botanical garden, where *L. dactylopii* is used as a natural enemy. The gardener of the botanical garden of Copenhagen (Anom., 2020a) mentioned *P. citri* as a common pest in the greenhouse. If Copenhagen uses *L. dactylopii* against *P. citri* and also insecticides, there is a risk that *P. citri* would start thriving even more.

As mentioned, *C. lycimnia* is a natural enemy of *S. coffeae* and *S. oleae* (Kullin, 2008), and it can be used effectively against *C. hesperidum* (Anom., 2020a). Using chemicals that kill *C. lycimnia*, might cause an increase in the abundance of *S. coffeae* and *S. oleae*, and other methods would have to be used to control them.

Since there is a 100 % mortality rate, there is a risk that the chemicals would affect other natural enemies, which were not tested and further research would be necessary in order to see other effects.

According to Suckling et al (2010), insecticidal baits on ants do affect non-target ants and they are not appropriate for at sensitive system. If the baits do affect non-target insects, then there is a possibility of it having negative effects on the ecosystem in the greenhouses. A species identification would be necessary, if the baits are species specific.

4.2 Rules and normal procedures of the botanical gardens

In the three botanical gardens included in this study, Aarhus, Copenhagen and Gothenburg, biological control is the method of plant protection, with the exception of Copenhagen and Gothenburg also using chemicals in exceptional cases.

All the botanical gardens point to new plant material as a source of new pests. Scale insects are easily transported with plants, and without proper inspection from the receivers, they can enter the greenhouses (Cranston & Gullan, 2014). Even if the scale insects are already present in the greenhouse, new arrival of the same species would be an extra source of spread inside the greenhouse.

A mechanical removal of *C. hesperidum* is possible and requires no means, other than technique and time. The gardeners need to be able to reach the affected locations on the plant and to get rid of the peeled-off scale insects in an appropriate way, so that they do not re-infest other plants. This method might damage the plant, and on a highly-infested plant, it might not be appropriate in regards to time management, and they are not sure to get rid of the pests.

Lang (2020, pers. comm.) states regarding to biological control, half of the time the praxis does not follow the theory. The example above of *C. montrouzieri* (see section 4.1) can be an indicator as to why biological control is not that straightforward, as it touches several trophic levels.

None of the botanical gardens listed pheromones treatment as control in their greenhouse. Pheromone treatment is mostly used on big crops, and no information on the use for greenhouses in botanical gardens is available. Pheromones would not defy the rules of the botanical gardens, because pheromones are not insecticidal.

A possible combination of 'Rope'-type dispensers and release of natural enemies, would be interesting to test. With ant exclusion, the natural enemies would have time to parasitize and keep the number of pests down. Ant identification might be necessary.

The use of chemicals would go against the botanical gardens rules and normal procedures of using biological control and affect other parts of their biological control program. The botanical gardens only use chemicals in exceptional cases, and baits could be suitable because of their low content of insecticides, if the infestation of *C. hesperidum* is classified as an exceptional case.

This study shows that the pests do not go away, continuous control is necessary, so it is important to find an easy and applicable way to treat it, when an attack is discovered. The treatment has to go in line with the rules and procedures of the botanical gardens and not disrupt the ecosystems in the greenhouses. Further research is needed on this issue.

There were some difficulties with the questionnaires and communication with the botanical gardens. Only two out of four botanical gardens (Aarhus and Gothenburg) replied to my questionnaire, and I only had short meeting and follow-up e-mails with the botanical garden of Copenhagen. The questions that were asked and answered was not the same for the three botanical gardens. It would have been optimal to visit the botanical gardens and speak with the gardeners in person several times, but this was not possible during the pandemic. Communication consisted mostly of e-mails and this can lead to misunderstandings.

5. Conclusions

- Proper cleaning in the greenhouses and inspection of new plant material is important to prevent the spread of *C. hesperidum* and arrivals of new aggressive ant species.

- Chemical control would not be suitable, since the botanical gardens have rules about only using biological control, except from exceptional cases, and chemicals would be detrimental to the ecosystems in the greenhouses.

- Insecticidal baits would be appropriate, if they are efficient and do not affects non-target insects.

- Ant exclusion can be necessary for the natural enemies of *C. hesperidum* to start parasitizing.

- Further research is needed on the subject of the effects of synthetic trail pheromone.

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Appendix 1

Plant protection with a focus on arthropods in greenhouses in botanical gardens.

1. Your name:

   - Oscar Björn & Åsa Kullin

2. Your title:

   - Gardener & gardener

3. Name of botanical garden:

   - Göteborgs Botaniska Trädgård

4. Who is responsible for plant protection in your botanical garden? (Profession)

   - The gardener and the garden workers are responsible for plant protection in their respective areas. However, Oscar Björn takes care of biological plant protection and Åsa Kullin is responsible for chemical control. Oscar and Åsa both work inside greenhouses and thus responds primarily with a focus on the greenhouse environment.

5. What is your overall plant protection strategy for arthropods?

   - We invest heavily in preventive measures through both the release of biological plant protection but also early intervention upon detection of infestation and sanitation. With regard to biological plant protection, we follow an annual schedule with release. Besides this, we release when needed. Physically acting agents are also widely used. We are constantly trying to review our growing conditions in order to make our collections be in the best condition as possible in the best possible way and thus be able to withstand various attacks. The goal is also for our gardeners to have knowledge of pests that occur in greenhouses. Discovered, identification and action. Chemical pesticides are used only in exceptional cases when there are no other alternatives.

6. What is the primary source of knowledge when finding new cases of arthropods?

   - Colleagues, supplier of biological plant protection but also through own searches in literature and the internet.

7. How do you document cases of attacks?

   - We have no collected documentation of our attacks. The respective gardeners get to know their departments and know associated problems.

8. How and where do you pass on your knowledge to other organizations and botanical gardens?

   - We have annual meetings with colleagues in our other botanical gardens where we discuss plant protection. We also pass on our knowledge to interns and groups on study visits.

9. Do you have any problems with new pests when you get new plant material?
   If so, which ones?

   - As a botanical garden, we come into contact with new plant material with regular intervals and thus also with various pests. It can be anything from mealybugs, scale insects to various mites.

10. Have you found any examples of arthropod attacks, where information about the specific arthropod was not available?
    If so, which ones?

    - As for the false spider mite, there is only a small amount of information available. We find it difficult to find effective measures against *Diaspis*.

11. Do you have any examples of plants that die because there was no solution to the attacks?

    - Not directly but plants become weakened over time. In *Amorphophallus*, there is a problem with bulb mites and in orchids, *Diaspis* is a problem.

12. Which arthropods do you use as preventive biological control against pests?

    - *Chrysoperla carnea*
    Cryptolaemus montrovieri
    Macrolophus pygmaeus
    Microterys nietneri
    Anagyrus fusciventris
    Leptomastix dactylopii
    Coccophagus scutellaris
    Encarsia citrina
    Encarsia formosa
    Amblyseius swirskii
    Amblyseius andersoni
    Neoseiulus cucumeris
    Phytoseiulus persimilis
    Orius majusculus
    Aphidius colemani
    Aphidius ervi
    Aphidoletes aphidimyza
    Eretmocerus eremicus

13. What arthropods do you use regularly when an attack occurs?

    - *Chrysoperla carnea*
    Cryptolaemus montrovieri
    Franklinothrips vespiformis
    Orius majusculus
    Macrolophus pygmaeus
    Coccophagus scutellaris
    Leptomastix dactylopii
    Anagyrus fusciventris
    Amblyseius swirskii
    Amblyseius andersoni
    Neoseiulus cucumeris
    Phytoseiulus persimilis
    Encarsia formosa

14. Do you have any examples of using alternative methods to use arthropods as biological control?

    - Do not know if we understand the question correctly. But we shoot predatory mites over the plants with a very small leaf blower to get a good spread.

15. I am interested in arthropods in botanical greenhouses, so if you have any exciting examples, feel free to share them with me.

    - Soft scales:
    Coccus hesperidum
    Saissetia coffeae

    Armored scale insects:
    Diaspis boisduvalii

    Mealybugs:
    Pseudococcus longispinus
    Pseudococcus viburni
    Planococcus citri

    Cockroaches:
    Pycnoscelus surinamensis
    Periplaneta Americana
    Periplaneta australasiae

    Mites:
    Tetranychus urticae
    Species of *Rhyzoglyphus*
    Species of *Phytonemus*

Ants:
Tapinoma sessile (Problematic in that they take care of and protect scale insects, mealybugs and aphids against certain natural enemies. This is due to the honeydew that they produce).

Then we have some woodlice that are problematic as they can go on e.g. orchid roots. It is unclear what species they are.

Plant protection with a focus on arthropods in greenhouses in botanical gardens.

1. Your name:

   - Bjørn Lang

2. Your title:

   - Greenhouse gardener

3. Name of botanical garden:

   - Væksthusene Århus v/Science museerne

4. Who is responsible for plant protection in your botanical garden? (Profession)

   - Bjørn Lang

5. What is your overall plant protection strategy for arthropods?

   - Biological control in the form of beneficials and insect soaps.

6. What is the primary source of knowledge when finding new cases of arthropods?

   - Borregård Bioplant. Books.

7. How do you document cases of attacks?

   - We do not have to document it, we detect an attack and react based on that. In addition, we have a release plan for beneficials for the whole year. Then we can prevent an attack as well as turn up or down in relation to the beneficials.

8. How and where do you pass on your knowledge to other organizations and botanical gardens?

   - We meet with other botanical gardens, zoos, greenhouses etc. We communicate to our guests - in everyday life.

9. Do you have any problems with new pests when you get new plant material?

   - Yes, scale insects and thrips.

10. Have you found any examples of arthropod attacks, where information about the specific arthropod was not available?
    If so, which ones?

- Yes, for example with thrips. Where a beneficial was supposed to attack it, but that didn't happen.

11. Do you have any examples of plants that die because there was no solution to the attacks?

    - Yes, severe attacks of spider mites, thrips and mealybugs can be particularly bad.

12. Which arthropods do you use as preventive biological control against pests?

    - We have many. Species of parasitoid wasps, ladybirds/larvae. Gallmidge larvae, predatory thrips, and we use predatory mites. Predatory bugs (assassin bugs).

13. What arthropods do you use regularly when an attack occurs?

    - It differs. We have many different pests.

14. Do you have any examples of using alternative methods to use arthropods as biological control?

    - Nothing concrete. Only primarily in relation to prevention and attack.

15. I am interested in arthropods in botanical greenhouses, so if you have any exciting examples, feel free to share them with me.

    - (no answer)