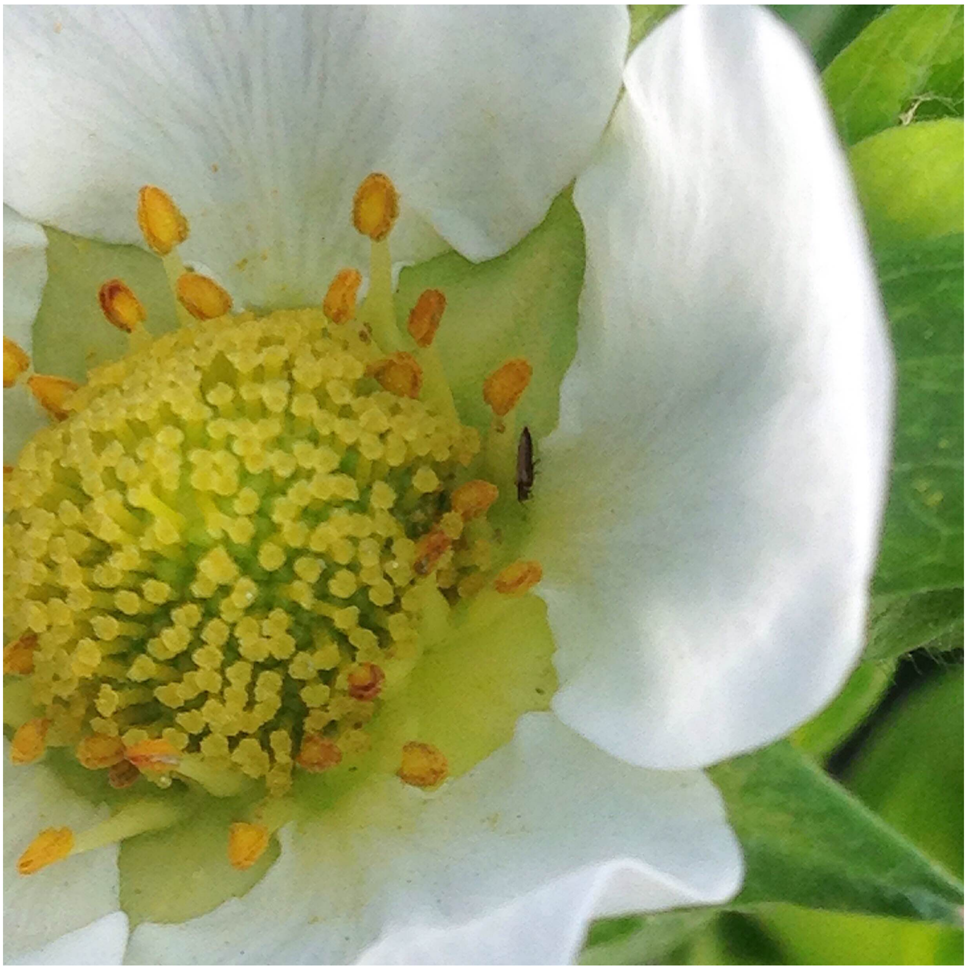
Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Landscape Architecture, Horticulture and Crop Production Science

Biological control of thrips with *Neoseiulus cucumeris* and *Orius majusculus* in strawberry tunnels

Biologisk bekämpning av trips med *Neoseiulus cucumeris* och *Orius majusculus* i jordgubbstunnlar

Robin Kalenius



Degree Project • 30 hec
Alnarp 2016

Biological control of thrips with *Neoseiulus cucumeris* and *Orius majusculus* in strawberry tunnels

Biologisk bekämpning av trips med *Neoseiulus cucumeris* och *Orius majusculus* i jordgubbstunnlar

Robin Kalenius

Supervisor:	Johan Stenberg, SLU, Department of Plant Protection Biology
Examiner:	Peter Anderson, SLU, Department of Plant Protection Biology

Credits: 30 hec
Project level: A2E
Course Title: Master's Thesis Project in Biology
Course Code: EX0800
Subject: Biology
Place of Publication: Alnarp
Year of Publication: 2016
Cover Art: *Robin Kalenius*
Online Publication: http://stud.epsilon.slu.se

Keywords: Thrips, *Neoseiulus cucumeris, Orius majusculus,* strawberry, tunnels, resistance, IPM, IGP

SLU, Swedish University of Agricultural Sciences
Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Plant Breeding

Acknowledgements

I am very grateful for those who have supported my work for this essay. Special thanks to my supervisor Johan Stenberg, SLU, Department of Plant Protection Biology with your support, feedback and knowledge. Big thanks also to Borregaard Bioplant ApS and Biobasiq, which made this possible by setting up contact, financing the project and delivering the biological agents and also very special thanks to my contact Nina Jørgensen at Borregaard Bioplant ApS, which has contributed with a lot of useful information during the experiment and answered all my questions. I would direct my appreciations to Eriksgården for their time and permission to perform the experiment in their tunnels. I am also very thankful for Peter Andersson SLU, Department of Plant Protection Biology, which attends as my examiner, Jan-Eric Englund, SLU, Department of Biosystems and Technology for the help with the statistics, Kajsa Ignell for the illustrations as well as all the support and last, appreciations to Salla Marttila, SLU, Department of Plant Protection Biology for all patience, input and help before and during the essay writing.

Abstract
Several cases of thrips resistance to pesticides have been observed due to heavy usage of insecticides during the seventies-eighties. Thrips complex enzyme metabolic detoxification system and fast reproduction has lead to a classification of major pest in strawberry production. This study evaluated a combination of two polyphagous biocontrol agents, the predatory mite *Neoseiulus cucumeris* and the minute predatory bug *Orius majusculus* and using the following research questions: Can the combination *Neoseiulus cucumeris* and *Orius majusculus* reduce thrips population in tabletop strawberry tunnels? This combination should theoretically target several thrips instars to reduce the thrips. The result showed a significant effect of biocontrol the last two weeks for total thrips and thrips on flowers. The experiment was terminated after five weeks due to threshold reached at experiment site and pesticides where applied. The result shows that biocontrol treatment could not completely control the thrips populations, but has an important potential as a part of IPM as it procrastinate pesticide usage. Additional experiments are required with biocontrol agents tested separately and in combination to analyze efficacy and evaluate reported intraguild predation (IGP) from *Orius* spp. on predatory mites, but also study the possible delayed insecticide resistance.

Sammanfattning
Flera fall av tripsresistens mot bekämpningsmedel har rapporterats som följd av den stora användningen av insekticider under sjuttio-åttiotalet. Tripsens komplexa enzym detoxifieringssystem och deras snabba reproduktion har lett till att de klassificeras som ett av de viktigare skadedjuren i jordgubbsodling. Denna studie testade en kombination av två biologiska bekämpningsagenter, rovkvalstret *Neoseiulus cucumeris* och den rovlevande skinnbaggen *Orius majusculus* utifrån forskningsfrågan: Kan kombinationen *Neoseiulus cucumeris* och *Orius majusculus* minska trips-populationer i jordgubbar på bord i tunnel? Denna kombination bör kunna predera på flera tripsstadier för att minska populationen. Resultatet visade en signifikant effekt av biologisk bekämpning de två sista veckorna för det totala antalet trips och antalet trips på blommor. Försöket avbröts efter fem veckor på grund av att tröskelvärdet hade uppnåtts och bekämpningsmedel tillämpades. Resultatet visar att biologisk bekämpning inte helt kunde kontrollera trips-populationer men det kan ha en viktig roll i integrerade växtskydds (IPM) program eftersom de kan senarelägga besprutningen av bekämpningsmedel. Ytterligare experiment behövs då biologiska bekämpningsagenter bör testas separat och i kombination för att utvärdera effektiviteten och den rapporterade intraguild predations (IGP) beteendet från *Orius* spp. på rovdjurskvalster, men också för att testa eventuell fördröjning av insektsresistens.

Content

1. Introduction

Thrips are widely spread through the world and are responsible for large damaging on agri- and horticulture crops (Reitz & Funderburk, 2012). Thrips are vectors for viruses in the *Tospovirus* genus, which can infect several ornamental and vegetable crops. Calculations from 1994 describe over one billion US dollars losses every year from the *Tomato Spotted Wilt Virus* (TSWV), which western flower thrips (WFT), *Frankliniella occidentalis* are the most important vector for. 12 % of the economic value from harvested tomatoes and peppers between 2001-2006 were lost due to thrips and TSWV (Reitz & Funderburk, 2012). Thrips are not known to be responsible for any virus on strawberry but are recognized as a major pest on them, especially in organic production (Jordbruksverket, 2013). The damage is often seen as bronzing spots (Jordbruksverket, 2013; UC IPM, 2010), and cracks with precipitation after thrips attacks (Jordbruksverket, 2013) and also aesthetical deformation on the fruit (Reitz & Funderburk, 2012). Thrips damages are known over whole Sweden and has become an increased problem since the nineties (Jordbruksverket, 2013). The problem will probably increase with climate change due to enhanced ability for thrips reproduction (Eckersten et al., 2008). The economic losses in strawberry production are yet not evaluated (Cluever et al., 2015) and seldom mentioned in literature (Reitz & Funderburk, 2012), but several pesticides are approved for use in Sweden (2016) as Calypso SC 480, Karate 2.5 WG and Raptol against thrips in strawberry production (Jordbruksverket, 2016). Due to increased spread of resistant cases against insecticides, thigmotactic behavior, which are the response of movements either towards or away from a contact of a solid body but also fast reproduction and several instars, should combinations of strategies be applied within integrated pest management (IPM) practices to manage thrips populations in strawberry production (Facun Sarmiento, 2014; Reitz & Funderburk, 2012). These IPM practices can be defined as "an ecosystem approach to crop production and protection that combines different management strategies and practices to grow healthy crops and minimize the use of pesticides" (FAO, n.d.). The big sections to maintain the sustainable practices for IPM are to prevent, monitor, responsible use when adapted to the crop along with several management strategies and finally follow-up (UC IPM, n.d.). The use of crops with modified expressing genes of the insecticidal crystal protein (Cry) *Bacillus thuringiensis* (*Bt*) has been a useful part in IPM, due to effective pest management and more environmental friendly substance than insecticides (Romeis, Shelton & Kennedy, 2008). But insect resistant cases have developed on some of these *Bt* crops as well (Liu et al., 2008). Biocontrol agents are one part of the IPM methods applied in crop production (Reitz &

Funderburk, 2012), it is a daily protection using predators or parasites (Pettersson & Åkesson, 2011). This IPM method has shown to have a part in delaying resistance on *Bt* crops (Liu et al., 2008). Reitz & Funderburk (2012) also describes biocontrol agents as an important part in IPM when "insecticide cannot function as a stand-alone control method".

Thrips

Thrips are often observed during warm-, dry- and windless conditions (Jensen, 2005; Pettersson & Åkesson, 2011). They are approximately one to three millimeters long with small, slim wings and short antennas (Jensen, 2005; Pettersson & Åkesson, 2011) and some species are apterous (Pettersson & Åkesson, 2011). They often appear in dark color, but differentiate in and between species and can have brighter color with whitish to yellow color (Facun Sarmiento, 2014; Jensen, 2005; Pettersson & Åkesson, 2011). Larvae and nymphs often show light colors (Facun Sarmiento, 2014; Pettersson & Åkesson, 2011). The most important thrips species are Western flower thrips (WFT) *Frankliniella occidentalis* (Thysanoptera: Thripidae) and has become an expanded problem in agri- and horticulture (Facun Sarmiento, 2014; Reitz, 2009) due to its spread of TSWV among other *Tospoviruses*, but also its reproduction potential and polyphagous diet (Facun Sarmiento, 2014; Reitz, 2009; Reitz & Funderburk, 2012). It was first detected in Netherlands 1983, for than become a large problem around Europe and rest of the world due to global international trading. The damage inflicted by thrips can often be observed through small dots in rows on the fruit or leaves but also deformation within the crop (Pettersson & Åkesson, 2011; Reitz, 2009). The thrips suck out the content of the plant cell, which later on could be seen as silvered or necrotic part called bronzing spots on the subject (Pettersson & Åkesson, 2011; Reitz, 2009). Coll et al. (2007) describe significant minimized strawberries receptacles with >10 thrips on the flower but also damages on stigmas and anthers. Light damages could be observed with five to ten thrips

FIGURE 1 Picture from measurement during 8.6.16 on strawberry, *Fragaria x ananassa* 'Charlotte' with bronzing damages inflicted of thrips. The picture also demonstrates presence of thrips larval in light orange color.

presence and two thirds of the strawberry affected with punctures and bronzing with more than fifteen thrips present (Coll et al., 2007). Thrips life cycle are described as egg; two larvae instars; two pupae instars (propupa & pupa; adult (Reitz, 2009). After 2-4 days (for *F. occidentalis*) eggs hatch (Reitz, 2009) and the two larval instars are feeding on crops when the two pupal instars have a non-feeding behavior, but are still mobile (Pettersson & Åkesson, 2011; Reitz, 2009). The pupal often hides in the soil (Pettersson & Åkesson, 2011; Reitz, 2009) but still a large number can remain on the crop (Reitz, 2009). The whole life cycle can be pulled off in nine to thirteen days with optimal conditions (28 degrees) for *F. occidentalis* (Reitz, 2009). Adult females oviposition on petioles, flower bracts, petals, into leaves and on developing fruit and *F. occidentalis* could with optimal temperature and capacities produce seven offspring a day and up to 200 in a lifetime. The oviposition and larval development could benefit through pollen feeding (Reitz, 2009). *F. occidentalis* males are reproduced through unfertilized haploid eggs, arrhenotokous parthenogenesis, and females through diploid fertilized eggs (Facun Sarmiento, 2014; Reitz, 2009). Because of its polyphagous diet, along with observation on over 250 different crops with a variety of approximately sixty families, it has a complex metabolizing enzyme detoxification system with a severe broad range of allelochemical-metabolizing genes (Reitz, 2009). The highly used doses of insecticides used in the seventies-eighties have lead to an increased resistance (Reitz, 2009), but thrips also have a thigmotactic behavior (Facun Sarmiento, 2014; Reitz, 2009; Reitz & Funderburk, 2012) and especially in strawberries hiding between sepals, which could create problems with the efficiency of contact insecticides. But also the low level of rotation between different insecticides is a problem, which occurs when fewer insecticides are approved for application (Reitz & Funderburk, 2012).

Biological control

Biological control is a pest management strategy using biological enemies for predation, parasitism or as pathogens (Pettersson & Åkesson, 2011). Several biocontrol agents are used for predation of thrips (Facun Sarmiento, 2014). Among these predators, mites are one of the largest groups of predators with species as *Neoseiulus cucumeris*, *Amblyseius degenerans, Amblyseius swirskii* among others. These groups often predate early instars of thrips (Facun Sarmiento, 2014). Introduction of *N. cucumeris* into greenhouse tomatoes production has showed an reduction of the thrips population to keep threshold levels intact (Shipp & Wang, 2003). *N. cucumeris* uses odors to find host plant or prey and can distinguish between and prefer crops (experiment for three plants in Solanaceae family) that are infested with *F. occidentalis* compared to undamaged, mechanical damage and pre-infested plants (Zhong et al., 2011). But in some cases the release of volatiles from damaged plants will not attract *N. cucumeris*, which Himanen et al. (2015) revealed in a study when bites from cyclamen mite and leaf beetle on young strawberry plants ('Polka' and 'Honeoye') didn't attract *N. cucumeris* in significant levels.

FIGURE 2 *Orius majusculus* predating on thrips in flower on tabletop strawberry (*Fragaria x ananassa* 'Charlotte') production. Picture captured during measurement on date 1.6.16.

Orius spp. (Hemiptera: Anthocoridae), often-called minute pirate bug is a common predator used due to its polyphagous feeding (Lattin, 1999). It is naturally abundant in nature (Bosco, Giacometto, & Tavella, 2008; Bosco & Tavella, 2013) and mainly feeds on thrips, mites, aphids, and whiteflies (Biobasiq, n.d.[1]). *Orius majusculus* are a known predator of thrips (Biobasiq, n.d.[1]) and inter alia used for commercial strawberry production. In an experiment did Bosco & Tavella (2013) release the biocontrol agent *O. majusculus* in tunnel strawberries (also sweet pepper in tunnel) to inventory the amount of *Orius* spp. during the season. They found significant more *Orius niger* than *O. majusculus* in the production of tunnel strawberries and sweet pepper. *O. majusculus* showed instead highest abundance in sweet leek fields and are described to be less adapted to high temperatures compared to *O. niger*, which would explain the absence (Bosco & Tavella, 2013). *O. laevigatus* is also a known predator of thrips in strawberry production in Portugal, where they have demonstrated reduced thrips population in caged experiments (Lattin, 1999). A study in management of *F. occidentalis* in sweet pepper production show that a release of less *O. laevigatus* could still effective manage predation with reduced thrips population (Weintraub, Pivonia, & Steinberg, 2011). But some experiment demonstrates different results when using *Orius* spp. for management method against thrips. Shipp & Wang (2003) revealed that *Orius insidiosus* failed to reduce thrips population when ten bugs/plant were released on one greenhouse tomato. Also could the combination of the predators lead to intraguild predation (IGP), when feeding on the other biocontrol agents instead of the pest. *O. insidiosus* and *A. degenerans* demonstrated this IGP behavior in greenhouse roses for predation of *F. occidentalis* (Chow, Chau, & Heinz, 2008). The result revealed that high densities of *A. degenerans* are more attractive for *O. insidiosus* than *F. occidentalis* (Chow, Chau, & Heinz, 2008). Also the combination with *O. laevigatus* and *A. swirskii* (Weintraub, Pivonia, & Steinberg, 2011) and *O. laevigatus* and *N. cucumeris* show IGP (Coll et al., 2007).

Aim of study

Due to increased cases of thrips resistance from insecticides (Reitz & Funderburk, 2012), it becomes more important to optimize biocontrol in crop production. *N. cucumeris* and *O. majusculus* are two potentially effective biocontrol agents against thrips, which seldom has been tested as combination in tabletop strawberry production, which this study will approach. The aim of this study is to answer the following research question:

Can the combination *Neoseiulus cucumeris* and *Orius majusculus* reduce thrips population in tabletop strawberry tunnels?

2. Material and methods

Site conditions

The experiment was performed at Eriksgården, Sjöbo (Sweden) (55°37'20.9"N 13°46'25.6"E), in nine tunnels (125 x 8.5 meter), constructed 2011. Grassland, farmland, berry tunnels and windbreaks surround the experiment tunnels (**FIGURE 3A**). Six rows are found in one tunnel, each row consists of a tabletop construction, which carries black plastic trays approximately one to one and a half meters above ground (**FIGURE 3B**). Each plastic tray (0.5 x 0.2 meter) contains four strawberry plants and the substrate used is peat, which are exchanged every year. Eriksgården use the Delta-T device WET Sensor to check pH, EC and temperature. Drippers together with a nutrition solution water the plants. Tunnels are ventilated and in order to not exceed 25°C.

Before the season, Eriksgården uses Karate against leaf rollers and hymenopterans and a pyrethroid and often Fastac 50 in the beginning of July (E-mail Anna-Karin Nilsson 2016-05-02). Between April-May and June, no pesticides are being used due to harvesting and remontating strawberries. In beginning of July there is a natural stop in the production and the strawberries will have another growth period, which is when they often could use pesticides against thrips and other insects if needed. The active substance in Fastac 50 is alpha cypermethrin (BASF, 2016), which are described as very harmful for both *N. cucumeris* and *O. majusculus* with 75 % population reduction (Koppert, n.d.) and Karate 2,5 WG with active substance lambda-cyhalothrin (Kemikalieinspektionen, n.d.), which could result in the same damage to *N. cucumeris* and *O. majusculus* populations as Fastac 50 (Koppert, n.d.).

Plants

Strawberry, *Fragaria x ananassa* 'Charlotte' (Rosaceae) grown and sold by De kemp, The Netherlands, were planted in nine tunnels at Eriksgården with four plants in each plastic tray. 'Charlotte' is a hardy remontating variety developed by Ciref in 2004 (Ciref, n.d.). The plant shows great resistance against phytophthora, and mildew but the fruit are less resistant. Yields are about 700-1200 gram per plant (Ciref, n.d.) and average weight of fourteen to sixteen grams per fruit (Ciref, n.d.; Öberg, 2008). It has good potential for tunnel production (Öberg, 2008) and is described as low nutrition demanding variety (Ciref, n.d.).

Biocontrol agents

Two types of biocontrol agents in combination were used for the study, *N. cucumeris* and *O. majusculus*. The predatory mite *N. cucumeris*, former name *Amblyseius cucumeris,* are delivered by Borregaard Bioplant ApS. It is polyphagous and used for young thrips and mites (Biobasiq, n.d.[2]) with a size of zero-point-five millimeters (Evergreen Growers, n.d.). Their lifecycle consist of three nymph instars and develops on approximately eight days to adult in twenty-five degrees and seventeen days in fifteen degrees (Biobasiq, n.d.[2]). They prefer high humidity and can normally lay fifty to hundred eggs as adult (Biobasiq, n.d.[2]). The introduction in the crop should be rather direct, when storage could have negative impact on the mites quality, but could be stored for a couple of days in temperature of ten to fifteen degrees (Biobasiq, n.d.[2]). The biocontrol agent is delivered in bags with 1000 mites containing all instars. *O. majusculus* are reared and distributed by Borregaard Bioplant ApS. They require long daytime and temperature between sixteen to thirty-four degrees humidity and are not that sensitive to low humidity (Biobasiq, n.d.[1]). *O. majusculus* are about three millimeters (Biobasiq, n.d.[3]) and the product contains wingless nymphs in second and third instar. The adult bug can lay about seventy-five eggs as adult that processes through five nymph instars (Biobasiq, n.d.[1]). This takes about twenty-five days in twenty degrees. The product should ideally be introduced in the crop directly, but could also be stored for three to four days in eight to ten degrees (Biobasiq, n.d.[1]). The biocontrol agents were either transported by car from Borregaard Bioplant ApS or sent through mail to Eriksgården and stored over the night. First transport was stored at Munketorpsgård over the night.

Treatment

The experiment was conducted in nine Haygrove pioneer 40 tunnels, with two treatments in each tunnel: Control (regular treatment) and Biocontrol (*N. cucumeris* and *O. majusculus*). The treatments were randomized through a draw with 50-50 choices and each treatment area (plot) was about 51 m^2 (6x8.5) and located ten meters into the tunnel (**FIGURE 3A**). The plot was marked in each corner with wrapping string and contained six rows with tabletops. Four strawberries were planted in each tray, and the trays were about zero-point-five meters long and zero-point-two wide. There was no other difference in the treatments of the plots than the added biocontrol agents. The tunnels are managed the same way with WET Sensor device, ventilation, nutrition and harvesting etc. In the biocontrol plots each row are supplied with three bags *N. cucumeris* and established with approximately two meters between (four plastic trays between). The first bag was placed in the beginning of the plot area, closest to the entrance and than every fourth plastic trays (approximately two meter) between. The bag containing *N. cucumeris* was attached to the base of the strawberry plant in the driest area. *O. majusculus* was delivered in small pipes with 500 *O. majusculus* in each pipe. *O. majusculus* were distributed equally over the nine plots with small amounts dropped three times on leaves and flowers one meter in front of *N. cucumeris* (The placement of biocontrol agents is shown in **FIGURE 3B**). Approximately 111 (=1000/9) *O. majusculus* were used for one plot and 18 (=111/6) on each row with three distributions, therefore 6 bugs/placement (=18/3). *N. cucumeris* bags were exchanged every fourth week and *O. majusculus* were supplemented every second week. The application of natural enemies followed instructions from Borregaard Bioplant ApS.

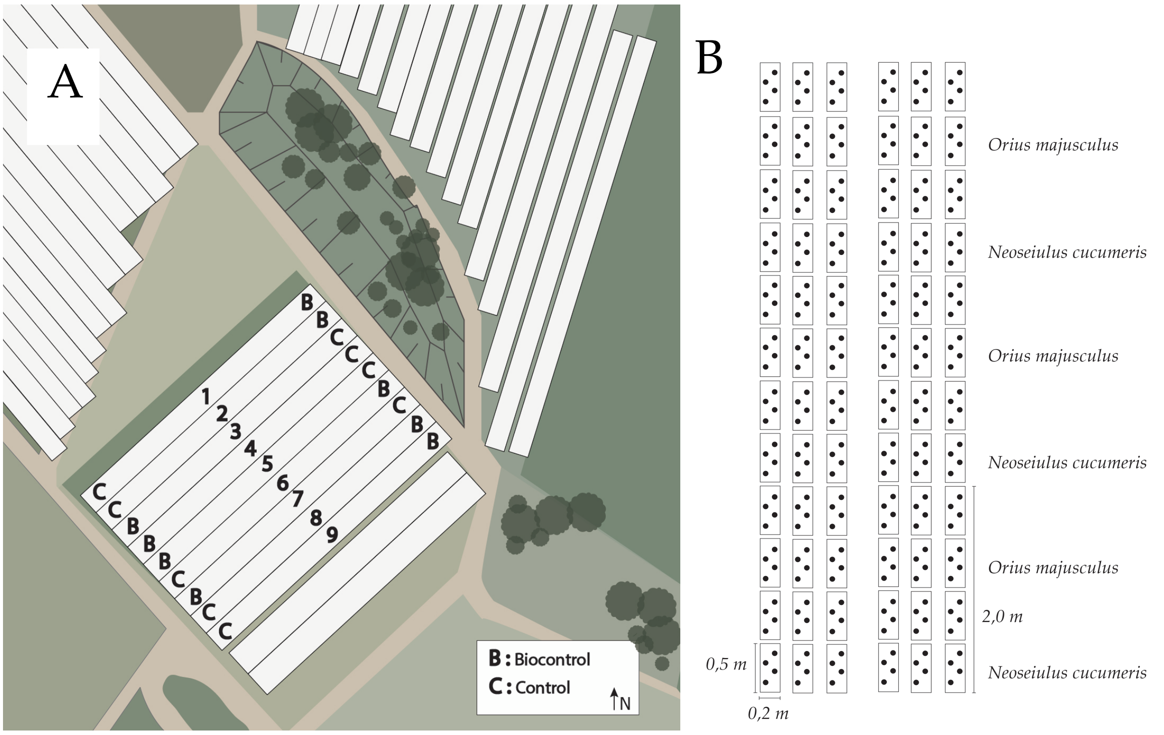


FIGURE 3 A Map of Eriksgården, Sjöbo (Sweden) (55°37'20.9"N 13°46'25.6"E) with nine strawberry tunnels (one tunnel are 125 x 8.5 meter). Tunnels are numbered from one to nine with two treatments in each tunnel; Control with regular treatment and Biocontrol with combination of *Neoseiulus cucumeris* and *Orius majusculus* to evaluate if biocontrol agents could reduce thrips in tunnels. The treatment plots are placed ten meters into each opening. **B** Each tunnel are built with six rows containing plastic trays on tabletops (one tray are 0.5 x 0.2 meter) with four strawberries planted in each tray, *Fragaria x ananassa* 'Charlotte'. The Biocontrol plot use combination of biocontrol agents *Neoseiulus cucumeris* and *Orius majusculus*. Three bags of *Neoseiulus cucumeris* were placed on each row every second meter and the treatment plot began ten meters into the tunnel closest to the entrance. *Orius majusculus* were distributed with six bugs every second meter beginning one meter in front of *Neoseiulus cucumeris* on each row. Total eighteen bags of *Neoseiulus cucumeris* and 111 *Orius majusculus* were used for one biocontrol treatment. The illustrations are not in correct scale. *Figure **A** and **B** were illustrated by Kajsa Ignell.*

Start date and placement of biocontrol 13.5.16 (week 0).

1. First measurement 18.5.16 (week 1).
2. Second measurement with additional supplying of *O. majusculus* 25.5.26 (week 2). Tunnels six to nine were without *O. majusculus* supplement due to unfortunate death of one of the pipes.
3. Third measurement 1.6.16 (week 3). Tunnels six to nine were supplied by one pipe (500 bugs) *O. majusculus*.
4. Fourth measurement with *O. majusculus* and *N. cucumeris* added 8.6.16 (week 4). Biocontrol plot in tunnel 3 and 4 were without new material of *N. cucumeris* due to missing bags in delivering.
5. Last measurement date and than further trials aborted when Eriksgården reached their threshold of thrips and pesticides were used on date 13.6.16 (week 5). The planned experiment should been finalized 4.7.16.

Sampling

The sampling process occurred once a week with not less than five days minimum and seven days maximum between each scoring. Each measurement was conducted between nine a.m. to seven p.m. For each sampling procedure, two plants from each row were randomly chosen. On each plant, three leaves, three flowers and three berries scored for thrips. If there was not enough sampling material on the strawberry, neighboring plants were used.

Data analysis

The effect of the biocontrol treatment on the number of thrips was tested using a generalized linear mixed model with a Poisson distribution fit by maximum likelihood (Laplace approximation) with the LME4 package in the statistical program R-3.3.1 for windows. The analysis used the treatment Biocontrol as a fixed factor and the tunnel as a random factor. The rows nested within tunnels. Syntax for the analyzes:

Model<-glmer(formula = Thrips ~ Treatment +(1|Tunnel/Row), family = poisson)

The model was used and analyzed for each week and tested total numbers and flowers, berries, leaves separately (**TABLE 1**). The model could only be performed during week 3 on leaves due to no thrips found to analyze.

3. Results

At week 0 thrips population was almost none, but increased during week 2 and with large increase from week 3 to week 4 (**FIGURE 4**), due to a high temperature. The thrips population was mainly observed on flowers (**FIGURE 5**). The thrips observed on flowers were often adults and found underneath and around the ovary and a thigmotactic behavior were observed during counting. A general lower average numbers of thrips were observed on berries (**FIGURE 6**). When carefully lifting the sepals on young and green berries thrips nymphs were often found in large numbers. Thrips were almost never observed on leaves (**FIGURE 7**). Significant difference between Biocontrol and Control were for total numbers weeks 2, 4 and 5 (**TABLE 1**). On flowers, significant differences were only found for week 4, 5 and on berries significant differences for week 2, 3 and 5 (**TABLE 1**). During week 5, berries scored higher average number in biocontrol treatment than in control treatment and showed a significant effect. The total thrips number was lower during week 5 compared to week 4. Thrips on leaves were only observed during week 3 and the numbers showed no significance.

TABLE 1 Results from generalized linear mixed model with a Poisson distribution fit by maximum likelihood (Laplace Approximation) evaluating effects of biocontrol (combination of *Neoseiulus cucumeris* and *Orius majusculus*) in strawberry tunnels. Start date 13.5.16 (week 0) and measurement dates 18.5.16 (week 1), 25.5.16 (week 2), 1.6.16 (week 3), 8.6.16 (week 4), 13.6.16 (week 5). No analysis could not be conducted for leaves on week 0, 1, 2, 4, 5 due to no thrips discovered. Significant effects in bold ($P < 0.05$)

Total

Week	Estimate	SE	P
0	1.099	1.155	0.341
1	1.204	0.658	0.067
2	-0.652	0.203	**0.001**
3	-0.138	0.103	0.198
4	-0.279	0.055	**3.89×10^{-7}**
5	-0.250	0.064	**8.73×10^{-5}**

Flowers

Week	Estimate	SE	P
0	30.250	3.702×10^{6}	1.000
1	24.130	226.510	0.915
2	-0.457	0.293	0.119
3	0.095	0.127	0.456
4	-0.321	0.064	**5.28×10^{-7}**
5	-0.201	0.072	**0.005**

Berries

Week	Estimate	SE	P
0	32.330	271.270	0.905
1	1.099	0.667	0.099
2	-0.823	0.283	**0.004**
3	-0.566	0.185	**0.002**
4	-0.160	0.106	0.133
5	-0.424	0.134	**0.001**

Leaves

Week	Estimate	SE	P
0	-	-	-
1	-	-	-
2	-	-	-
3	-	1.414	1.000
4	-	-	-
5	-	-	-

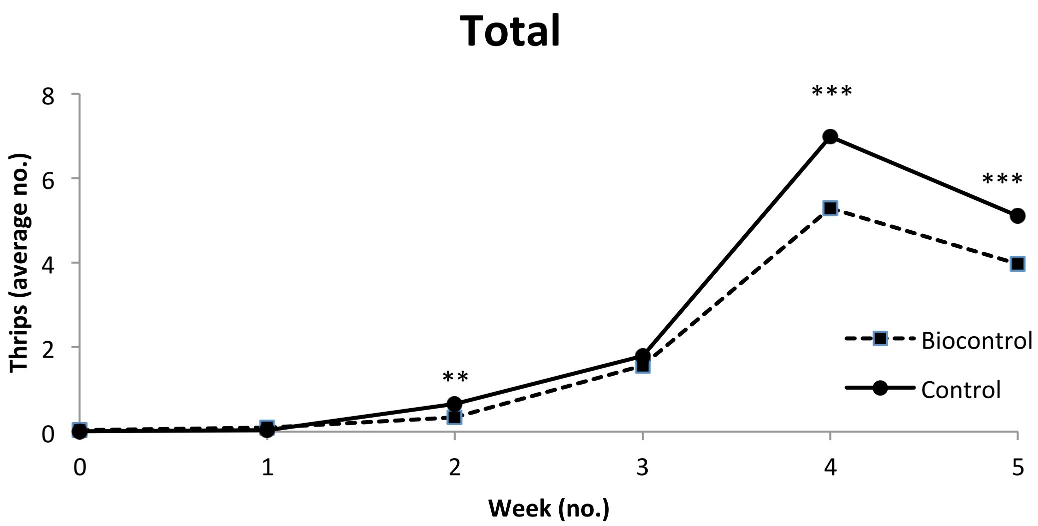


FIGURE 4 Average numbers of thrips from start date 13.5.16 (week 0) and measurement dates 18.5.16 (week 1), 25.5.16 (week 2), 1.6.16 (week 3), 8.6.16 (week 4), 13.6.16 (week 5). Significant difference marked with asterisk * (P < 0.05); ** (P < 0.01); *** (P < 0.001). The dashed line represents experimental plots treated with biocontrol agents *Neoseiulus cucumeris* and *Orius majusculus*.

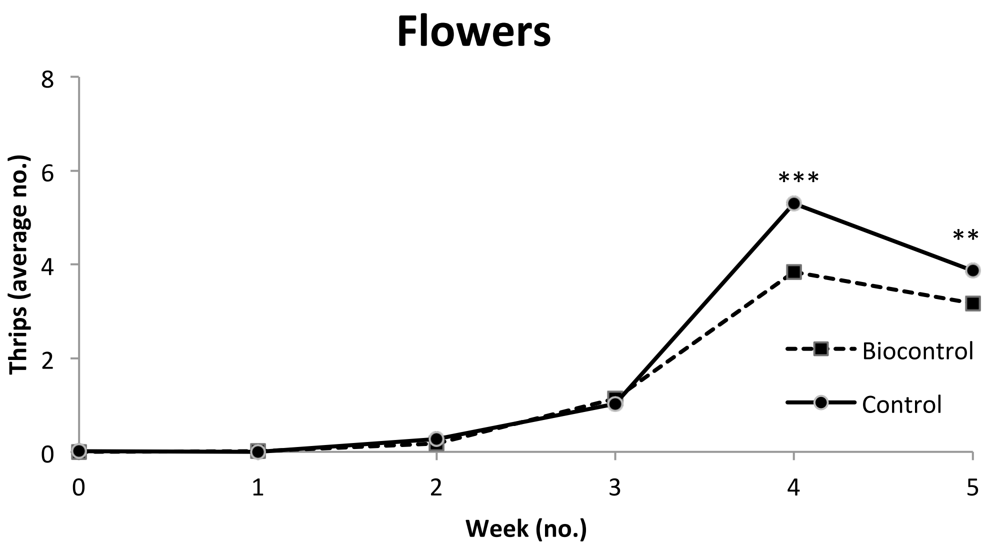


FIGURE 5 Average numbers of thrips from start date 13.5.16 (week 0) and measurement dates 18.5.16 (week 1), 25.5.16 (week 2), 1.6.16 (week 3), 8.6.16 (week 4), 13.6.16 (week 5). Significant difference marked with asterisk * (P < 0.05); ** (P < 0.01); *** (P < 0.001). The dashed line represents experimental plots treated with biocontrol agents *Neoseiulus cucumeris* and *Orius majusculus*.

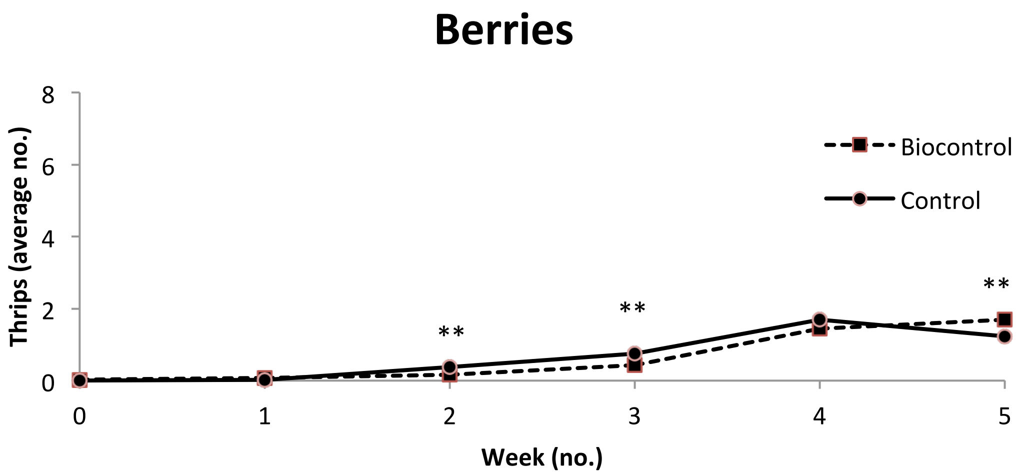


FIGURE 6 Average numbers of thrips from start date 13.5.16 (week 0) and measurement dates 18.5.16 (week 1), 25.5.16 (week 2), 1.6.16 (week 3), 8.6.16 (week 4), 13.6.16 (week 5). Significant difference marked with asterisk * (P < 0.05); ** (P < 0.01); *** (P < 0.001). The dashed line represents experimental plots treated with biocontrol agents *Neoseiulus cucumeris* and *Orius majusculus*.

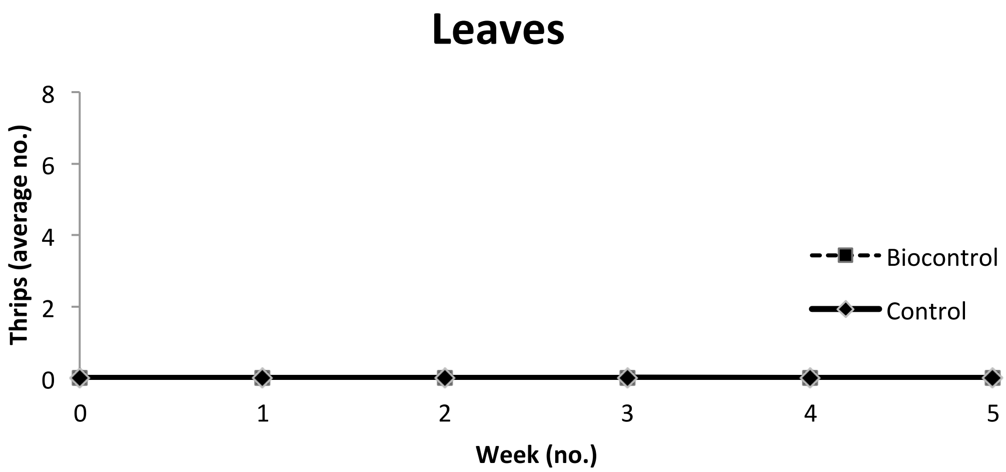


FIGURE 7 Average number of thrips from start date 20160513 (week 0) and measurement dates 20160518 (week 1), 20160525 (week 2), 20160601 (week 3), 20160608 (week 4), 20160613 (week 5). The two lines overlap through the whole figure. Significant different marked with asterisk * (P < 0.05); ** (P < 0.01); *** (P < 0.001). The dashed line represents experimental plots treated with biocontrol agents *Neoseiulus cucumeris* and *Orius majusculus*.

4. Discussion

The results of the completed study reveal significant effects of biocontrol using a combination of *N. cucumeris* and *O. majusculus* on thrips in strawberry tunnels. But the average difference of thrips number between the both treatments is probably not sufficient to completely control the thrips but could procrastinate insecticide use. Biocontrol can be an important part of IPM programs, which will work as a preventive pest management tool. Studies also point to that biocontrol agents can delay pest counter-resistance to *Bt* crops (Liu et al., 2008). Those cases could theoretically have the same effect on resistant development from insecticides. Thrips resistance has existed since seventies-eighties when heavy densities of insecticides were used (Reitz, 2009) and Reitz & Funderburk (2012) describes that the increased resistant cases also are dependent on a low rotation time between insecticides. But also that fewer insecticides being approved for the market (Reitz & Funderburk, 2012). In Sweden there are three insecticides approved for against thrips in strawberry production, Calypso SC 480, Karate 2.5 WG and Raptol (Jordbruksverket, 2016). With only three approved insecticides, biocontrol makes a severe important part in IPM-programs. The economical factors are not evaluated in this study but speculations could be interesting for observing the value of biocontrol agents. With insecticide use follows a waiting time with probable losses of non-harvested and non-sold crops. The effectiveness of insecticide could be decreased with the evolution of resistance, but also with thrips thigmotactic behavior and the grower use a contact insecticide. The cost of biocontrol agents depends on species and application intensity. Different combinations of agents will differ in cost and efficacy; therefore studies should be continued within biocontrol treatments to find a sufficient treatment with right intensity and efficacy with low cost to control thrips populations.

The tunnels at Eriksgården are positioned close to a grassplot (**FIGURE 3A**) and the surroundings are also covered with farmlands, which are managed during the season. This probably discompose thrips habitat and direct them to the closest tunnel (Tunnel 1, see **FIGURE 3A**), which are reported as the most exposed one (Rickard Bergkvist 2016-04-04). The trays with substrate and strawberry plants in the tunnel are exchanged every year and the ground is covered with black plastic mulches. This probably results in mostly overwintering thrips in the environment around the tunnels. During the experiment adult thrips were often seen in flowers and thrips nymphs on green fruit. The oviposition probably occurred on strawberry flowers, which than egg hatch on early-developed fruit. The available pollen in the flowers may be a useful food source for thrips, which they could advantage from with beneficial oviposition and larval development (Reitz, 2009). The result of thrips adult in flowers could be the consequence of pollen feeding. To manage thrips thigmotactic behavior, *N. cucumeris* were used as biocontrol agent. *O. majusculus* were used for all thrips instars. Due to difficult application and side openings of tunnels for ventilation, incidences of wet *N. cucumeris* bags were observed, which could result in decreased protection against thrips from the predatory mites. There is also a known IGP shown from *Orius* spp. on predatory mites (Chow, Chau, & Heinz, 2008; Weintraub, Pivonia, & Steinberg, 2011, which we don't know how it effected the result. But during each measurement there was low presence of *O. majusculus* observed, which than will make it questionable in which scale the result was affected by IGP. Some unpredictable incidents occurred during the experiment as delayed application of *O. majusculus* and missing bags of mites, which could have inflicted the result. The result still gives us indications on a significant effect of the biocontrol agents. What still remains unanswered is the result of thrips population on berries week 5 (**FIGURE 6**). The result showed significantly more thrips on berries in control plot compared to berries on biocontrol plot. But remarkable for the measurement was a cold day with a strong wind present in the tunnels, which could have an impact on thrips habitat behavior. One theory could be that larval and pupas could have completed their instars to adult thrips that often were found on flowers. Still the low average amount of thrips on berries should not have any larger importance when the threshold for light damages are not received referring to Coll et al. (2007). The focus of thrips on flowers is more essential in strawberry tunnels, which stood for almost the whole total thrips population (**FIGURE 5**).

Conclusion

This experiment can conclude a significant difference between the two treatments, that the combination of *N. cucumeris* and *O. majusculus* can reduce thrips population in tabletop strawberry tunnels. For future studies the biocontrol agents should be evaluated over several years, separate and in combination to see effectiveness, but also evaluate if IGP behavior can be observed due to several reported cases on *O*. spp. and predatory mites. The biocontrol treatment is a sufficient part in IPM programs for thrips because of the increased presented resistant cases. If biocontrol can delay insecticide resistance as Liu et al. (2014) reported for *Bt* crops, it should be crucial and imperative tool for further management. The biocontrol treatment for this experiment is not fully sufficient to completely control thrips, but it can procrastinate insecticide use.

References

BASF (2016) *Fastac 50*. Available at: http://www.agro.basf.se/agroportal/se/sv/special_1/product_catalogue_1/product_details_1771.html [2016-06-14]

Biobasiq (n.d.[1]) *Orius-rovskinnbagge*. Available at http://www.biobasiq.se/produkter/nyttodjur/orius.aspx [2016-08-05]

Biobasiq (n.d.[2]) *Tripsrovkvalster (Amblysieus (korrekt släkt-namn; Neoseiulus) cucumeris*). Available at: http://www.biobasiq.se/produkter/nyttodjur/amblyseuis-cucumeris.aspx [2016-08-05]

Biobasiq (n.d.[3]) *Orius mot trips*. Available at: http://www.nyttodjur.se/produkt/orius-mot-trips.aspx [2016-08-09]

Bosco, L., Giacometto, E., & Tavella, L. (2008). Colonization and predation of thrips (Thysanoptera: Thripidae) by *Orius* spp. (Heteroptera: Anthocoridae) in sweet pepper greenhouses in northwest Italy. *Biological Control*, *44*(3), 331–340. http://doi.org/10.1016/j.biocontrol.2007.10.027

Bosco, L., Tavella, L., & others. (2013). Distribution and abundance of species of the genus *Orius* in horticultural ecosystems of northwestern Italy. *Bulletin of Insectology*, *66*(2), 297–307.

Coll, M., Shakya, S., Shouster, I., Nenner, Y., & Steinberg, S. (2007). Decision-making tools for *Frankliniella occidentalis* management in strawberry: consideration of target markets. *Entomologia Experimentalis et Applicata*, *122*(1), 59–67. http://doi.org/10.1111/j.1570-7458.2006.00488.x

Chow, A., Chau, A., & Heinz, K. M. (2008). Compatibility of *Orius insidiosus* (Hemiptera: Anthocoridae) with *Amblyseius (Iphiseius) degenerans* (Acari: Phytoseiidae) for control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on greenhouse roses. *Biological Control*, *44*(2), 259–270. http://doi.org/10.1016/j.biocontrol.2007.11.002

Ciref (n.d.) *Charlotte*. Available at: http://ciref-agriculture.fr/varietes-fraises-ciref/charlotte/ [2016-07-14]

Cluever D. J., Smith A. H., Funderburk E. J., Frantz G. (2015). *Thrips in Florida strawberry crops*. Entomology and Nematology Department, UF/IFAS Extension.

Eckersten, H., et al. (2008). *Bedömningar av klimatförändringars effekter på växtproduktion inom jordbruket i Sverige*. Uppsala: Department of Crop Production Ecology, Swedish University of Agricultural Sciences.

Evergreen Growers (n.d.) *Amblyseius cucumeris rapid release*. Available at: http://www.evergreengrowers.com/amblyseius-cucumeris-2974.html [2016-08-08]

Facun Sarmiento, H. K. (2014). *Integrated pest management of western flower thrips*. Retrieved from http://dspace.library.uu.nl/handle/1874/293882

FAO (n.d.) *AGP-Integrated pest management*. http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/ipm/en/ [2016-08-02]

Himanen, S., Vuorinen, T., Tuovinen, T., & Holopainen, J. K. (2005). Effects of cyclamen mite (*Phytonemus pallidus*) and leaf beetle (*Galerucella tenella*) damage on volatile emission from strawberry (*Fragaria* × *ananassa* Duch.) plants and orientation of predatory mites (*Neoseiulus cucumeris* , *N. californicus,* and *Euseius finlandicus*). *Journal of Agricultural and Food Chemistry*, *53*(22), 8624–8630. http://doi.org/10.1021/jf050676j

Jensen K. (2005). *Kompendium om ekologisk odling av jordgubbar*. Länsstyrelsen Västra Götalands Län.

Jordbruksverket (2013). *Risk- och konsekvensanalys för jordgubbar – insekter*. Strategin för växtskyddsmedel PM. Jordbruksverket

Jordbruksverket (2016) *Växtskyddsmedel 2016 – Bär*. Alnarp: Växtskyddscentralen, Alnarp, jordbrukverket

Kemikalieinspektionen (n.d.) *Karate 2,5 WG*. Available at: http://webapps.kemi.se/BkmRegistret/Kemi.Spider.Web.External/Produkt/Details?produktId=8987&produktVersionId=8995 [2016-06-15]

Koppert (n.d.) *Side effects*. Available at: https://www.koppert.com/side-effects/?tx_koppertsideeffects_beneficialeffect%5BbiologyId%5D=1&cHash=405b4af879b3f0b2a154dce065977544 [2016-06-14]

Lattin D. J. (1999) Bionomics of the Anthocoridae*. Annual Review of Entomology 1999. 44:207–31*

Liu, X. *et al.* (2014) Natural enemies delay insect resistance to *Bt* crops. *PLoS ONE* 9, e90366, doi:10.1371/journal.pone.0090366

Öberg, E. (2008). Nytänkande i Norrbottnisk bärproduktion. *Journal Nr.: 2007-3291-Delrapport*, *2*. Retrieved from http://hushallningssallskapet.se/wp-content/uploads/2015/06/nytankande-i-norrbottnisk-barproduktion-2008.pdf

Pettersson, M-L & Åkesson, I. (2011) *Trädgårdens växtskydd*. Korotan: Natur och kultur. ISBN 978-91-27-13070-8

Reitz, S. R. (2009). Biology and ecology of the western flower thrips (Thysanoptera: Thripidae): The making of a pest. *Florida Entomologist*, *92*(1), 7–13. http://doi.org/10.1653/024.092.0102

Reitz, S. R., & Funderburk, J. (2012). *Management strategies for western flower thrips and the role of insecticides*. INTECH Open Access Publisher. Retrieved from http://www.academia.edu/download/30777999/2012_Reitz_Funderburk_01.pdf

Romeis J, Shelton AM, Kennedy GG (eds.) (2008) *Integration of insect-resistant genetically modified crops within IPM programs*. e-ISBN 978-1-4020-8373-0. Springer

Shipp, J. L., & Wang, K. (2003). Evaluation of *Amblyseius cucumeris* (Acari: Phytoseiidae) and *Orius insidiosus* (Hemiptera: Anthocoridae) for control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on greenhouse tomatoes. *Biological Control*, *28*(3), 271–281. http://doi.org/10.1016/S1049-9644(03)00091-4

UC IPM (n.d.) *What is integrated pest management (IPM)?* http://www2.ipm.ucanr.edu/WhatIsIPM/ [2016-08-02]

UC IPM (2010) *Strawberry*. Available at: http://ipm.ucanr.edu/PMG/r734301211.html [2016-07-18]

Weintraub, P. G., Pivonia, S., & Steinberg, S. (2011). How many *Orius laevigatus* are needed for effective western flower thrips, *Frankliniella occidentalis*, management in sweet pepper? *Crop Protection*, *30*(11), 1443–1448. http://doi.org/10.1016/j.cropro.2011.07.015

Zhong, F., He, Y.-R., Gao, Y., Qi, G.-J., Zhao, C.-Y., & Lu, L.-H. (2011). Olfactory responses of *Neoseiulus cucumeris* (Acari: Phytoseiidae) to odors of host plants and *Frankliniella occidentalis* (Thysanoptera: Thripidae)–plant complexes. *Arthropod-Plant Interactions*, *5*(4), 307–314. http://doi.org/10.1007/s11829-011-9135-6

Unpublished references
Anna-Karin Nilsson E-mail 2016-05-02

Rickard Bergkvist 2016-04-04