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Catching the European tarnished plant bug, *Lygus rugulipennis* (Hemiptera: Miridae), using baited funnel traps

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Preface:

First of all I would like to thank my supervisor Nina Trandem for great help during the whole process of this thesis, making it a positive experience.

I would also thank Michelle Fountain, Bethan Shaw and all other I met at East Malling Research for the warm welcome and a great visit. And for letting me use the results from the studies I contributed in for my thesis.

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For further information see: www.coreorganic2.org.

Abstract:

The European tarnished plant bug, *Lygus rugulipennis*, (Hemiptera: Miridae) is a highly polyphagous species considered a pest on numerous crops, among these strawberry causing malformed or “cat-faced” berries. No effective biocontrol agents are commercially available against this pest. To contribute in the development of traps for controlling *L. rugulipennis* in organic strawberry crops three studies investigating several aspects influencing catches were done in South-eastern Norway and in the UK. In the first study unbaited collision traps and funnel traps enhanced with green vanes and synthetic *L. rugulipennis* sex pheromone and/or a synthetic strawberry plant volatile (“PV2”) were set up and emptied fortnightly through most of the growth season in five strawberry fields (in Akershus, Norway). The plant volatile is a known attractant to another strawberry pest (the strawberry blossom weevil, *Anthonomus rubi*) and a possible attractant to *L. rugulipennis* as well. The sex pheromone was not found attractive to the new generation of *L. rugulipennis* in Norway. When PV2 was present less *L. rugulipennis* were caught. Compared to traps in semi natural/boundary habitat, the catches were significantly higher in strawberry crops. The PV2 attracted *A. rubi* as expected but not early in the season. In the second study one funnel trap baited with the *L. rugulipennis* sex pheromone blend was filmed for three days (in Kent, UK). All *Lygus* visiting the trap were recorded. Only between 7 and 18% were confirmed to be captured, all of them walking on the vanes for some time. The third study was to investigate the application of a slippery substance (Fluon) to the funnel would increase the trap efficiency of traps without cross vanes. The traps coated with fluon caught more than the control traps, but the difference was not significant on a 0.05 level. The traps did not seem to attract pollinators to any extent. To further develop the trap for higher catches the trap design has to be improved to increase the percentage of visiting *L. rugulipennis* to get caught.

Index:

	Page:
Preface:.....	i
Abstract:.....	ii
Index:.....	iii
1. Introduction:.....	1
1.1. <i>Lygus rugulipennis</i> :.....	3
1.2. Objectives:.....	5
2. Material and methods:.....	5
2.1. Study area efficacy trial:.....	5
2.2. Experimental design:.....	9
2.3. Collection methods:.....	9
2.3.1. Funnel traps:.....	10
2.3.1.1. PV2:.....	10
2.3.1.2. <i>Lygus rugulipennis</i> sex pheromone.....	10
2.3.2. Collision traps:.....	11
2.3.3. Tap sampling:.....	11
2.3.4. Sweep netting:.....	12
2.4. Damage assessment:.....	12
2.5. Pesticide application:.....	12
2.6. Behaviour study:.....	13
2.7. Fluon trial:.....	14
2.8. Statistics:.....	14
3. Results:.....	15
3.1. Efficacy trial:.....	15
3.1.1. Trap phenology:.....	17
3.1.2. Trap and lure differences:.....	17
3.1.3. Habitat differences:.....	18

3.1.4. Nymph catches:.....	18
3.1.5. Sex ratios:.....	18
3.1.6. Strawberry damage:.....	19
3.1.7. <i>Anthonomus rubi</i> catches:.....	19
3.1.8. Pollinator catches:.....	20
3.2. Behaviour study:.....	21
3.2.1. Time spent on trap:.....	21
3.2.2. Time of activity:.....	21
3.2.3. Trap efficacy:.....	22
3.2.4. How they were caught:.....	23
3.3. Fluon trial:.....	23
4. Discussion:.....	24
4.1. Catch phenology:.....	24
4.2. Habitat differences:.....	25
4.3. Nymph catches:.....	25
4.4. The lack of females:.....	26
4.5. Strawberry damage:.....	26
4.6. <i>Anthonomus rubi</i> catches:.....	27
4.7. Pollinator catches:.....	27
4.8. Trap efficacy (UK):.....	27
4.9. Fluon trial:.....	29
5. Conclusion:.....	30
6. References:.....	31
7. Appendix:	35

1. Introduction

Fighting pests is a crucial part of increasing yields and income of farmers of all crops. So far the most used way to fight pests has been pesticides, which often kill insects useful to the crops as well, such as pollinators and predatory insects. The use of pesticides also leaves residue on the fruit. Baker et al. (2002) found that across 8 fruits and 12 vegetable crops, 73% of the samples grown conventionally showed residue of pesticides. In some crops, including strawberries, the percentage of samples with pesticide residue was more than 90%. A report from Bioforsk and the Norwegian Food Safety Authority (2012) stated that 95% of the sampled strawberries produced in Norway had pesticide residues. To find alternative methods to fight pest species would be preferable, especially for organic growers who are not able to use pesticides.

The strawberry (*Fragaria × ananassa*) has been cultivated on a big scale in the temperate regions of the world since the early 1800 (Cross et al. 2010). Of arthropods, mollusks and nematodes, more than 90 species are known pests of strawberry, around 10 of these are considered to be serious economic pests (Cross et al. 2010). In Norway, strawberry is mainly produced during the summer and the varieties used are almost exclusively June-bearing varieties (Korona: 45%, Senga S. 20%, Polka 15%, Honeoye 10%) (Davik et al. 2000). The total acreage of strawberry production in Norway in 2013 was just over 1500 hectares (Haslestad, 2014). In Norway the biggest strawberry market is fresh fruits, with only 20% of the production going to the processing industry (Davik et al. 2000). According to “Frukt- og grønnsaksgrossistenes servicekontor” a total of 9 417 tonnes of strawberries were imported to Norway in 2012, most of which from Belgium. In 1999 the import of strawberries to Norway were less than 2200 tonnes (Davik et al. 2000), showing a great increase in import of strawberries in Norway during the last few years.

The European tarnished plant bug, *Lygus rugulipennis*, is considered to be a serious pest in strawberry crops across Europe, especially in late season crops (Easterbrook, 2000; Fitzgerald and Jay, 2011). No effective biocontrol agents are commercially available against this pest, making the growers reliant on pesticides (Fitzgerald and Jay, 2011). No predators have been shown to be able to keep the damage level from *Lygus* species down during high migration levels (Cross et al. 2010).

There have been tested several alternative strategies of controlling the *L. rugulipennis* and related species either without any use of pesticides or in combination with pesticides, both in

strawberry and other crops, among these are “trap cropping”. Trap cropping is a method where the crop plant is interplanted with a plant species more attractive to the pest species to lure the pest away from the crop. Stern et al. (1969) greatly reduced the number of *Lygus* spp. in cotton fields interplanted with alfalfa (*Medicago sativa*) compared to control fields. However, Easterbrook and Tooley (1999) found that strawberry fields interplanted with *M. sativa* did not reduce the number of *L. rugulipennis* in the strawberry crops in the UK. They also found that strawberry fields surrounded with a barrier of *Matricaria recutita* delayed the buildup of *L. rugulipennis* in the strawberry crop, but there was no consistent reduction of *L. rugulipennis* populations. According to Swezey et al. (2007) interplanting alfalfa into an organic strawberry field did not reduce the numbers of the Western tarnished plant bug (*Lygus hesperus*), unless the strips of alfalfa were regularly vacuumed with a tractor mounted vacuum machine.

Another alternative to pesticides is mass trapping. Mass trapping is a method of pest management using species-specific chemicals, such as host- and mate location chemicals or aggregation chemicals, to attract insects into traps (El-Sayed et al, 2006). The chemicals may be emitted by a plant preferred by the insect, or other food source or host (food or host location chemicals) (Koczor et al. 2012), or by the insect itself (sex pheromones, aggregation pheromones), (Yasuda, 1995). Both sex pheromones and aggregation pheromones have been shown to have potential in monitoring and control of numerous species (Burkholder, 1984; Millar et al, 2002; Yasuda, 2005; Yasuda and Higuchi, 2012). Different trap types are utilized in combination with the attractants, such as sticky traps (Yasuda et al, 2007), delta traps (Zhang and Aldrich, 2003) and funnel traps (Yasuda 1995, Ross and Daterman 1997, Switzer et al. 2009). Funnel traps consist of a bucket, and a funnel to prevent insects to escape after they are caught. Other components, such as vanes, can be fitted to funnel traps to increase catches of some species. Baited with an attractant, traps like these might reduce the numbers of insects or the damage caused by them.

Developing mass trapping traps targeting more than one pest species may be of great economic importance for growers of crops struggling with multiple pest species.

The project, “Softpest Multitrap”, the Eranet, Core Organic aims to develop a funnel trap targeting both the Strawberry blossom weevil, *Anthonomus rubi*, and the European tarnished plant bug, *L. rugulipennis*, in strawberry and *A. rubi* and the Raspberry beetle (*Byturus*

tomentosus) in raspberry crops, for monitoring and mass trapping, giving organic growers an alternative to control or monitor these species.

1.1. *Lygus rugulipennis*

L. rugulipennis is a true bug in the family Miridae (Fig.1). It ranges from 4.7-5.7 mm in length and vary greatly in color (Skipper, 2013). It is found throughout the Palaearctic region and is also considered to be naturally holarctic, after specimen believed to be *Lygus perplexus* were re-identified as *L. rugulipennis* (Schwartz and Sudder, 1998). *L. rugulipennis* is a highly polyphagous species. Varis (1972) reported it to feed on more than 100 plant species from over 30 families, but has later been reported to utilize at least 387 different species from 57 families as host plants (Skipper, 2013). Among these many are economically important plants such as



Fig. 1: Picture of *Lygus rugulipennis* (Photo: Nina Trandem).

lettuce, alfalfa (Accinelli et al. 2005), pine, sugar beet, clover (Varis, 1972). Taksdal and Sørum (1971) recorded that *L. rugulipennis* also damage strawberry. The species was not considered to be a significant pest on strawberry in other countries such as the UK until considerable fruit losses were attributed to *L. rugulipennis* in cultivars with late season fruits in the early 1990's (Easterbrook, 2000). *L. rugulipennis* is now considered a serious pest in strawberry in the UK as well, especially in late season crops (Easterbrook, 2000; Fitzgerald, 2011), where in some cases more than 50% of the berries may be downgraded (Fitzgerald, 2011)

In Norway and the rest of northern Scandinavia *L. rugulipennis* act as a univoltine species (Varis, 1972), while it in other European countries it may go through more than one generation a year. It is reported to go through 1-2 generation a year in Denmark (Skipper, 2013) while it in England go through 2-3 generations a year (Fitzgerald, 2011).

L. rugulipennis overwinters as adults mainly in the substrate of coniferous forests (Varis, 1971), but have in the UK been found over-wintering in strawberry fields (Easterbrook, 1997). The adults migrate to surrounding, open fields such as strawberry fields in May-June where it reproduces (Varis, 1971). However in the UK where *L. rugulipennis* go through more

than one generation the over-wintering generation tends to reproduce in various weed species before the second generation migrate and reproduce in strawberry crops (Easterbrook, 1997).

L. rugulipennis cause damage on strawberry by feeding on the strawberry flowers and developing berries causing malformed, or “cat-faced” berries (Jay et al. 2004). The damage are mainly caused by nymphs and adults hatched from eggs laid in the strawberry crop (Fitzgerald, 2011).

Malformation of strawberries is caused by damaged, or a lack of fertilized achenes (Taksdal and Sørum, 1971). For strawberries to grow the ovules contained in the achenes must be fertilized (Nitsch, 1950). The fertilization of one ovule is sufficient to cause growth, through the release of the plant hormone “auxin”, in the area surrounding that achene, and the weight of a strawberry are positively proportionate to the number of fertilized achenes (Nitsch, 1950). Nitsch (1950) also found that if achenes are removed from the strawberry the growth will stop immediately, thereby showing that the achenes control the growth of the surrounding tissue during the whole development. Handley and Pollard (1993) found that *L. lineolaris* primary feeding sites on strawberry flowers were the achenes, causing considerable damage to the achenes fed upon. However there are several other factors than feeding by *Lygus* species that may cause damage to achenes, such as insufficient pollination, pollen sterility, frost or fungicides (Sørum & Taksdal, 1970, Taksdal and Sørum, 1971).

L. rugulipennis males have previously been shown to be attracted to components of a sex pheromone emitted by females (Innocenzi et al. 2004, Innocenzi et al. 2005). However little is known about the attraction phenology of this sex pheromone blend in countries where *L. rugulipennis* go through only one generation each year. It would be preferred to have a lure that also attract females of the species. The most relevant possible attractant to test in this thesis is a odour resembling a plant volatile emitted from wild strawberry flowers (*Fragaria vesca*) already used in the Softpest project to attract both sexes of *A. rubi*.

The damage caused to strawberry by *L. rugulipennis* is reported to vary between different varieties of strawberries. Labanowska (2007) found a damage level from 1% to 53% in different strawberry varieties. Easterbrook and Simpson (2000) found that the strawberry variety “Bolero” had significantly lower number of misshapen fruits than other everbearing varieties. Rhains and English-Loeb (2003) report that the phenology and productivity of the strawberry plant influence the feeding and economic impact of *Lygus lineolaris* more than what cultivare do, however Dale et al. (2008) found that wild strawberry (*Fragaria*

virginiana) was more resistant to *L. lineolaris* damage than cultivated strawberry (*F. × ananassa*)

1.2. Objectives

The overlying objective of this thesis was to contribute to the development of traps for controlling *L. rugulipennis* and *A. rubi* in organic strawberry crops. Specifically the following aspects of trapping *L. rugulipennis* were investigated:

- The catch phenology of baited traps placed in both crop and boundary/semi natural habitats in Norway, using more neutral monitoring methods as a comparison.
- Comparison of traps with *L. rugulipennis* sex pheromone blend and PV2, alone and in combination with respect to catch of *L. rugulipennis* of the two sexes.
- Presence of nymphs in the crops and in the traps, and the amount of berry damage
- Side catches of pollinators (bees), do any of the lures or the trap itself attract pollinators
- The behavior of *L. rugulipennis* on the trap, and the efficacy of the current trap + lure design.
- The effect of making parts of the trap more slippery (with Fluon).

2. Material and methods

This thesis consists of three separate experiments. The field work for the lure efficacy experiment was carried out in South-eastern Norway, while the behavior study and the “Fluon” experiments were done at the grounds of East Malling Research, UK

2.1. Study area efficacy trial

The efficacy experiment was carried out in 10 sites, 6 in strawberry crops, and 4 in boundary/semi-natural sites, all located in Ås and Frogn counties in South Eastern Norway. A description of the sites are shown in table 1.

Table 1: Description of the 10 sites used in the experiment in Norway.

Site	Habitat	Start date	End date	Surroundings
Saxebøl Rv 152 Crop 1	Strawberry Crop Korona Planted 2010	25.04.2013	18.07- 2013	Fig. 3 Bordering another Strawberry field, oat field, a small area of coniferous, and a private garden.
Saxebøl Rv 152 Crop 2	Strawberry Crop Korona Planted 2012	25.04.2013	27.09.20 13	Fig. 3 Strawberry fields on three sides. Oat field on last side.
Saxebøl Farm Crop 1	Strawberry Crop Korona Planted 2011	25.04.2013	15.08- 2013	Fig. 3 Strawberry fields on three sides. Forth side it border a barn/bare soil
Saxebøl Farm Semi-natural	Semi-natural	25.04.2013	27.09.20 13	Fig. 3 Newly clear-cut spruce forest. The clear-cut border to spruce forest on two sides an older clear-cut on one side. On the fourth side it border to a oat field.
Saxebøl Farm Crop 2	Strawberry Crop Florence Planted 2012	25.04.2013	27.09- 2013	Fig. 3 Border to Strawberry fields on two sides, an oat field and spruce forest.
Saxebøl Rv152 Semi- Natural	Semi-natural	25.04.2013	27.09- 2013	Fig. 3 Clear-cut spruce forest. Early succession. Border to spruce forest on one side. To a gravel road on one side and further clear-cut on the last two sides.
Kirkejordet Crop	Strawberry of Various cultivares	27.04.2013	07.11.20 13	Fig. 4 Border to an area of bare soil on two sides, A grass-lawn on one side, and a small experimental plot of spruce on the fourth side.
Kirkejordet Semi-Natural	Semi-natural	27.04.2013	07.11.20 13	Fig. 4 High grass, <i>Urtica</i> species, <i>Lupinus</i> species. Border to a road on two sides. Grass lawn and a small forested patch.
Huseby Semi- natural	Semi-natural	01.05.2013	27.09.20 13	Fig. 5 Small semi-forested island between a Wheat field and a road.
Huseby Crop	Strawberry Crop Korona Planted 2011	01.05.2013 (06.06.2013)*	27.09.20 13	Fig. 5 Border to cereal fields, a potato field and a private garden. Because another experiment was conducted in this field early in the season there was only one funnel-trap baited with the <i>L. rugulipennis</i> sex-pheromone were deployed in the start of the season as a monitor trap.

*Until this date only *L. rugulipennis*-monitor trap was placed out.

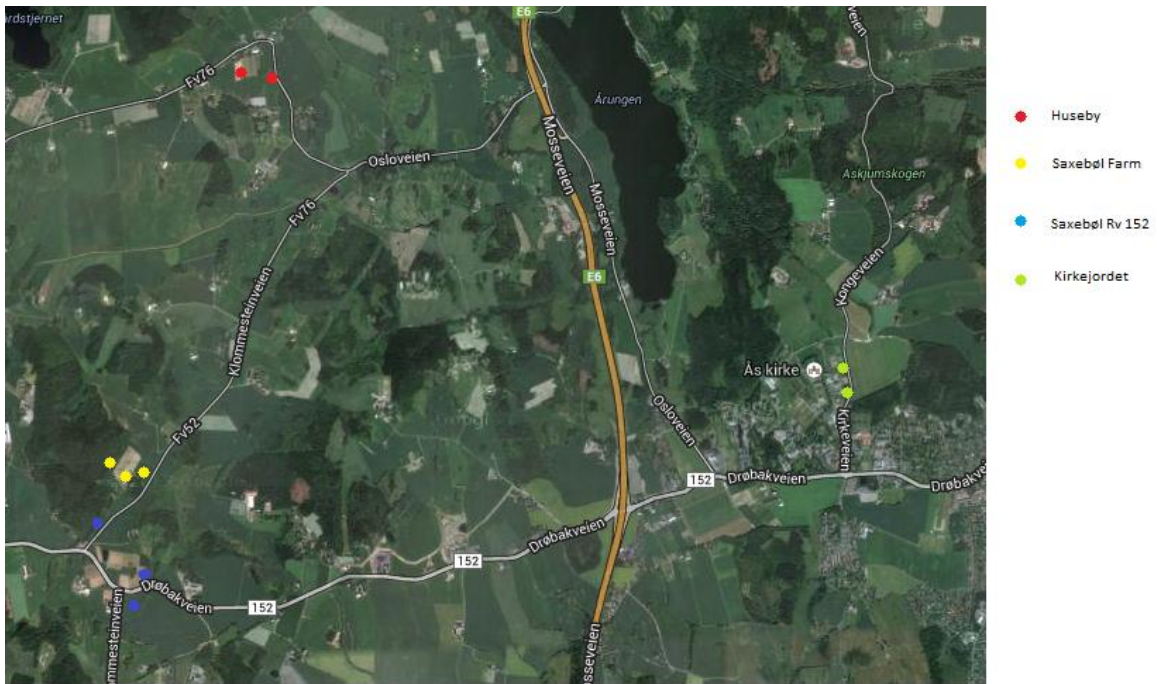


Fig. 2: Map showing the placement of all 10 sites (Ås/ Frogn, Norway).

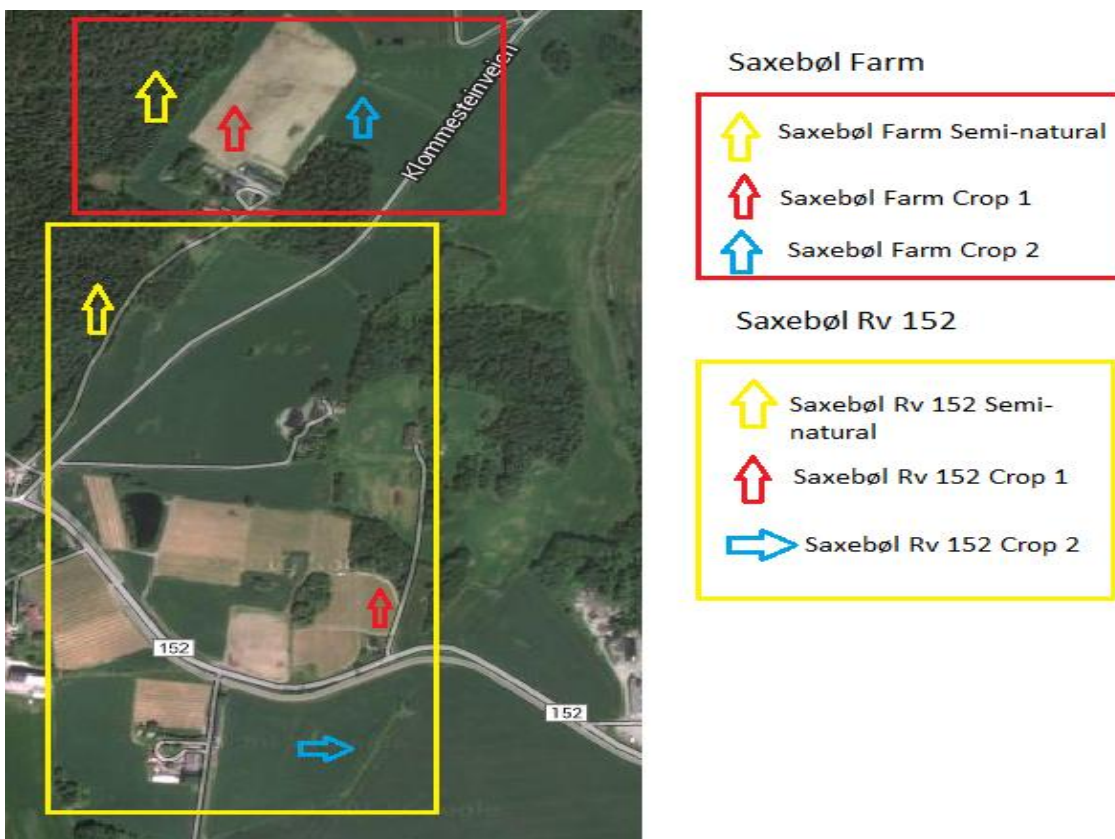


Fig. 3: Detailed map of the “Saxebøl Farm” (Red square) and “Saxebøl Rv 152” (Yellow square) sites. Arrows indicate trap stations. Direction of arrow indicates the direction of the trap configuration, shown in fig. 6, with collision traps at the base of the arrow. The “Saxebøl Farm” area consisted of a total of about 3.5 ha of strawberry, while the “Saxebøl Rv 152” area consisted of about 8.5 ha strawberry crops.



Fig. 4: Map over “Kirkejordet” Arrows indicate trap stations. Direction of arrow indicate the direction of the trap configuration (Fig. 6) with collision traps at the base of the arrow.



Fig. 5: Map over “Huseby” Arrows indicates trap stations. Direction of arrow indicate the direction of the trap configuration with collision traps at the base of the arrow (Fig. 6). The size of the strawberry field was 0,6 ha

2.2. Experimental design

Five traps were placed in a fixed pattern (Fig. 6) in each of the sites through most of the growth season of 2013 (Table.1). Each trap station consisted of two collision traps and three funnel traps with lures. One funnel trap was baited with the female *L. rugulipennis* sex pheromone blend, one with the strawberry plant volatile (PV2) an attractant to *A.rubi*. The third funnel trap was baited with both the *L. rugulipennis* sex pheromone and PV2. The trap configuration is shown in fig.6. The position of the three lure combinations in the funnel traps was randomized in each site. Some of the sites were too small to fit the distances shown in fig.6. In site 7 (Kirkejordet Strawberry) the distance between the two collision traps, and between the funnel traps were reduced to 3,6m,. The distance between the collision traps and the funnel traps were kept at approximately 15m. In site 8 (Kirkejordet Semi-natural) and 9 (Huseby Semi-natural) the distance between the funnel traps were reduced to 9m.

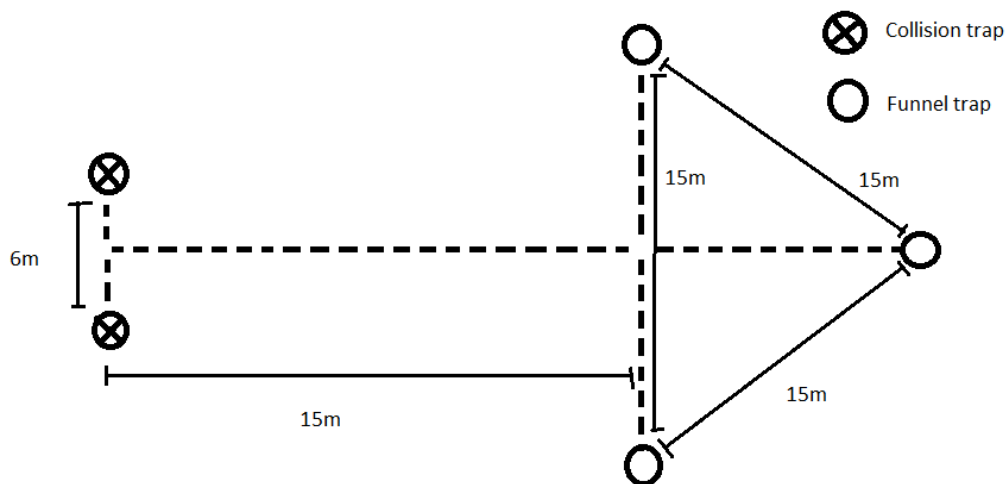


Fig.6 :Trap configuration at each site. Some of the distances were reduced in some of the sites.

2.3. Collection methods

Both passive and active collection methods were used in this experiment. An active collection method is when you actively attract a species to the trap, in this case the funnel traps. A passive collection method is a collection method with no form of attractant, where you therefore get a random sample of the insect fauna in the collection site; this consisted of the collision traps, the tap sampling and the sweep-netting.

All traps were emptied every 14 days. From the funnel traps all insects (spiders excluded) were collected in coffee-filters, marked with trap number and site, and brought into the lab. From the collision traps, where the insect catches tended to be higher the samples were roughly sorted in field, keeping anything that could resemble Mirids, or the *A. rubi* as well as bees and bumblebees. All specimen of *L. rugulipennis* and *A. rubi* were recorded and sexed.

2.3.1. Funnel traps

The funnel traps (Fig.7) consisted of a bucket to collect the insects, a funnel with a 10cm wide opening at the entrance and 3cm wide opening in the bottom, cross vanes and a lid to prevent rain falling into the trap. The funnel traps were baited with two different lures in this experiment. The lure was placed in a basket placed in a hole in the middle of the lid, so it was extended into the middle of the cross-vanes. In the combination traps the *L. rugulipennis* pheromone had to be taped to the bottom of the lid with the tip pointing towards the middle because both lures did not fit in the basket. The traps were fastened to the ground using a one meter long wire. The cross-vanes were 12cm wide at the bottom, 14cm wide at the top and 11cm high. The whole trap including the cross vanes were green. The area of the cross vanes, or the “catch area” was a little over 210 cm². Both lure types were changed monthly.



Fig.7:Funnel-trap placed in strawberry crop.

2.3.1.1. PV2

PV2 is a synthetic emulation of a plant volatile extracted from wild strawberry flowers (*Fragaria vesca*). It is attractive to the strawberry blossom weevil (*A. rubi*). The content of the PV2 is confidential, and will therefore not be mentioned in this paper.

2.3.1.2. *Lygus rugulipennis* sex pheromone

The *L. rugulipennis* sex-pheromone is a synthetic chemical composition resembling a pheromone produced by *L. rugulipennis* females to attract males during the mating season. The blend is made up of three components, Hexyl butyrate, (E)-2-hexenyl butyrate and (E)-4-Oxo-2-hexenal in the rate 100:3:20 (Innocenzi et al. 2004; Fountain et al. 2014)

2.3.2. Collision traps

Collision traps were made up of two plates of 3mm thick clear plexi-glass mounted in a cross, further description in fig. 8 and placed in plastic boxes approximately 21cm across the diagonal, and 8 cm high. The boxes were covered in black plastic (Fig. 8) to prevent pollinators to be attracted to the white plastic the boxes were made of. The “catch area” of this trap was approximately 1160 cm².

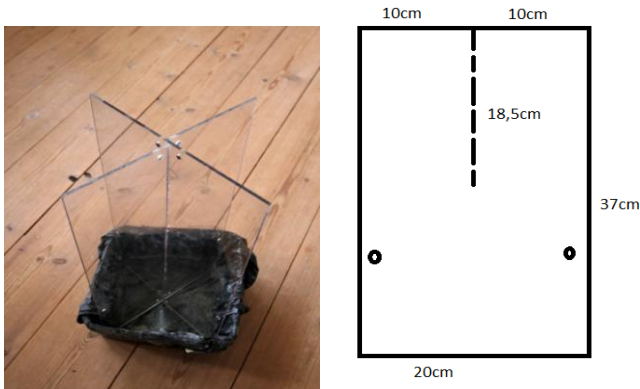


Fig.8: Measures of plates of collision traps, and the collision trap mounted. 37cm high and 20cm wide mounted in a cross by cutting an 18,5cm slice in the middle of the 20cm side of the plexi-glass. (Photo: Ida Gundersen)

2.3.3. Tap sampling

Tap sampling was done to sample mirids, adult and nymphs, in the strawberry crops.

Strawberry plants were gently shaken for 5sec over a 45cm round white tray with a 60 sector removed (Fig. 9). An exhaustor with a 6mm tube was used to collect the insects from the tray, all true bugs and weevils as well as other insects fitting in the 6mm wide tube of the exhaustor were collected. Each tap sampling consisted of insects collected from 50 strawberry plants. The plants were randomly selected from the area between the traps.



Fig. 9: Tray used in the tap sampling

2.3.4. Sweep netting

In the semi-natural habitat sweep netting was done instead of the tap sampling. Sweep netting was not used in strawberry crops because it would damage the plants, and the plant height and density make it difficult to do the sweep netting. It was done using a 50cm butterfly-net swept in a figure “8” along three line transects of approximately 20m each. An exhaustor was used to collect the insects from the net. This was done between each of the line transects. All true bugs (Adult and nymphs) and insects that might resemble Mirids, as well as all weevils that could fit through the tube of the exhaustor were collected.

After both the tap sampling and the sweep netting the insects collected were put in 70% ethanol for storage, and brought into the lab.

Both the tap sampling and the sweep-netting were done at least once every month from the first treatment on the 21th of June and until the 19th of August.

2.4. Damage assessment

Assessment of the amount of malformed berries was carried out in all crops. This was done just before the harvest started in the respective field. All ripe or ripening berries on random strawberry plants were assessed until at least 100 berries were checked. All the plants that were counted were in the area between the traps. The damage on the berries was scored from one to four where one is no damage, two is slight damage, three is severe damage and four is completely malformed.

2.5. Pesticide applications

The saxebøl sites there were sprayed with pesticides twice. At the “Saxebøl Rv 152” sites and the “Saxebøl Farm Crop 1” site it was sprayed with the Pyrethroid “Karate” at May 21th, and with the chloronicotyinyl “Calypso” at the 22nd of June. In the “Saxebøl Farm crop 2” it was sprayed with “Karate” the 3rd of June and with “Calypso” the 10th of July. At the “Huseby crop” it was sprayed once with “Calypso” at the 2nd of June.

2.6. Behaviour study

This study was conducted in a weedy field at East Malling Research, Kent, UK (Fig. 10).



Fig. 10: Site of behavior study and fluon trial, East Malling Research, UK

The experiment was done by filming a funnel trap of the same type as the one described in the earlier experiment to see *Lygus* bugs approach and land on, or fall in, the trap.

The camera was a CCD box cctw camera with Sony super HAD CCD sensor fitted with a V6x17 17-102mm Canon tv zoom lens. It was connected to a wireless transmitter, and stored on a hard drive

The funnel trap wwas placed on the ground and baited with the *L. rugulipennis* sex pheromone.

The behavior of all *Lygus* visiting the trap was recorded, as well as whether or not the *Lygus* was caught and how long it stayed on the trap. Also what time of the day the Mirids approached the trap.

The recordings used in this trial were filmed from 16th of August until 18th of August.

Because of a lack of light during the night the recordings was not possible to analyze from around 20.30 to about 06.00.

2.7. Fluon trial

This experiment was conducted in the same weedy field as the behavior study (Fig. 10). The same type of traps as in the previous experiments was used in this experiment, however the cross vanes was removed. Traps were put up 20m apart around the edge of the weedy field, approximately along the red square shown in fig. 10. The trap setup is shown in fig. 11. Every second trap the funnel was coated with Fluon, and the rest were control traps not coated with Fluon. All traps were baited with the three component *L. rugulipennis* sex-pheromone. Fluon (polytetrafluoroethylene), is a fluoropolymer that when applied in the funnel make the surface more slippery.

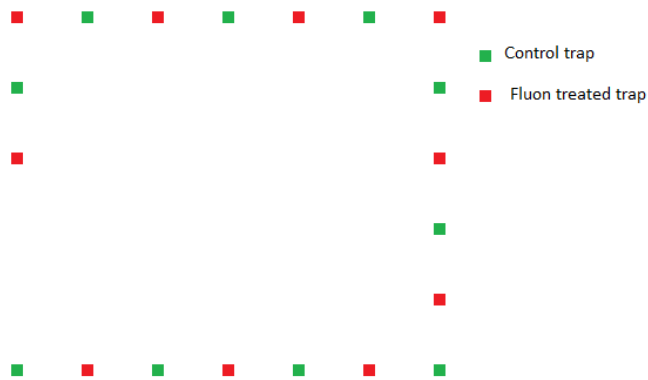


Fig. 11: Trap configuration in the Fluon trial. The space between each trap was 20m. Traps placed approximately along the red square shown in fig. 10.

2.8. Statistics

The statistical program “Minitab 16” was used for all statistical analyses in this thesis.

The sum of all male *L. rugulipennis* catches from each baited funnel trap was transformed (using Log base 10) to homogenize the variance. A general linear model was used to test for differences between treatments and habitats. Because of very low catches the data from the collision traps and funnel traps baited with only PV2 was excluded from the *L. rugulipennis* data. The data from the “Huseby crop” was also excluded because the *L. rugulipennis* monitor trap (with sex pheromone) had no competition during the peak catch. *A. rubi* data were treated the same way, however in this case only the two treatments containing PV2 was included, and the data from “Huseby crop” was included except for the time the *L. rugulipennis* monitor trap was the only trap.

To test the difference between the fluon treated traps and the control, both a paired t-test and a One-way ANOVA were done. All data were transformed (using Log base 10) to homogenize the variation. To see if the effect of the fluon wore off only the fluon data were used in a One-way ANOVA, testing for difference between the two collection dates. The same was done with the control data to see if the same trend as with the fluon data was seen.

A 95% confidence interval for the probability of a visiting *Lygus* to get caught was made using the following formula:

$$[\hat{p} \pm z_{\alpha/2} \times SE(\hat{p})] \text{ where } \hat{p} = \frac{\text{Lygus caught}(X)}{\text{Visiting Lygus}(n)}, \text{ and } SE(\hat{p}) = \sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

3. Results

3.1. Efficacy trial

The catches of *L. rugulipennis* at each trap station are shown in fig.12. The highest catches of *L.rugulipennis* were found in the funnel traps baited with the sex-pheromone and the combination traps (Fig. 12). A total of 376 *L. rugulipennis* were caught during the trial.

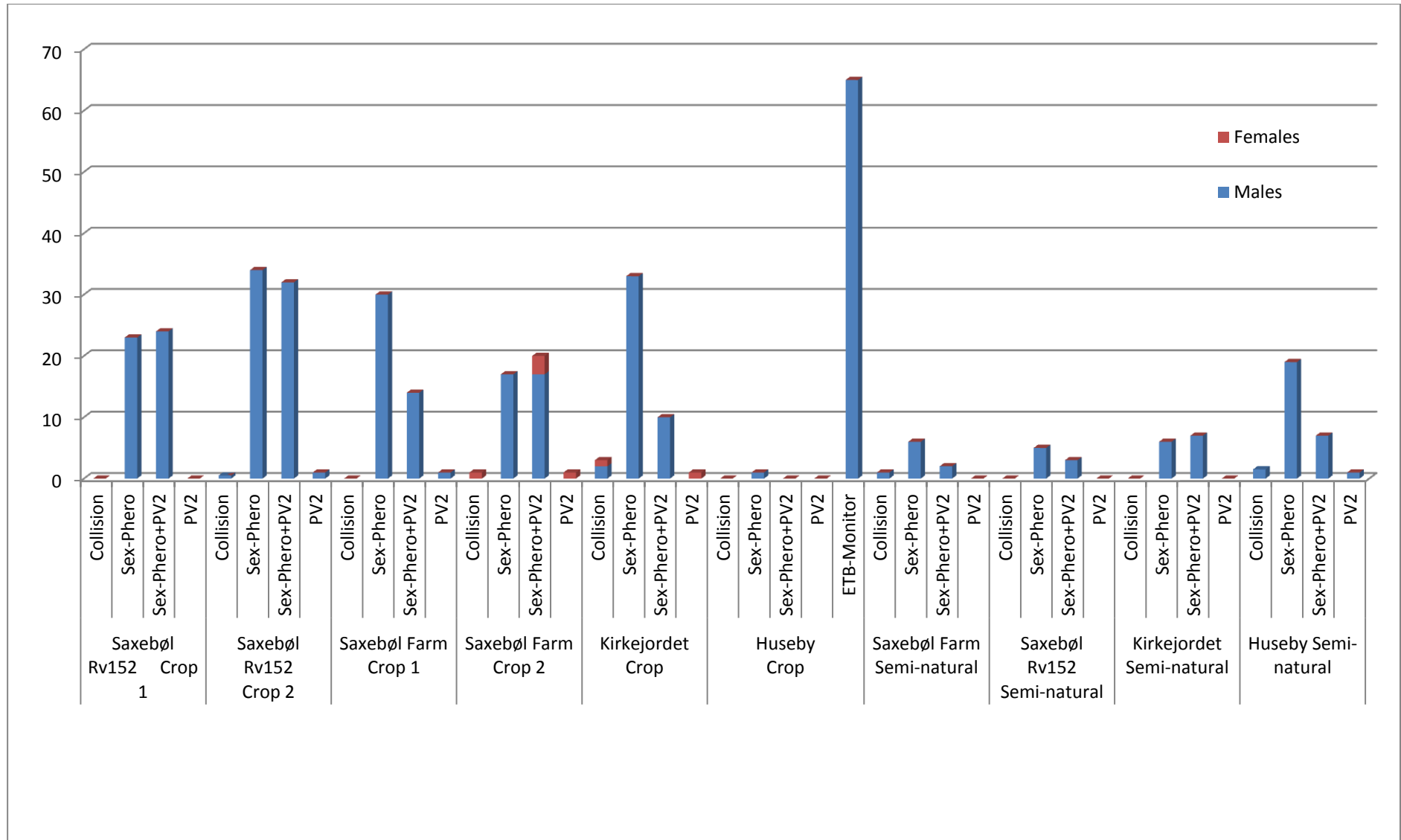


Fig. 12: Total number of *L.rugulipennis* caught per trap at the different trap-station.

3.1.1. Trap phenology

The catches started immediately after trap deployment (fig. 13). In the crops the catch peaked at the first collection date (09.05.2013), or during the first two weeks of the trap deployment in both the *L. rugulipennis* sex-pheromone traps and the traps baited with both lures. In the semi-natural habitat the catches peaked at the next collection date (23.05.2013), or during the next two weeks. After this the catches of *L. rugulipennis* declined fast, and only 22 of the 376 *L. rugulipennis* were caught after June 20th.

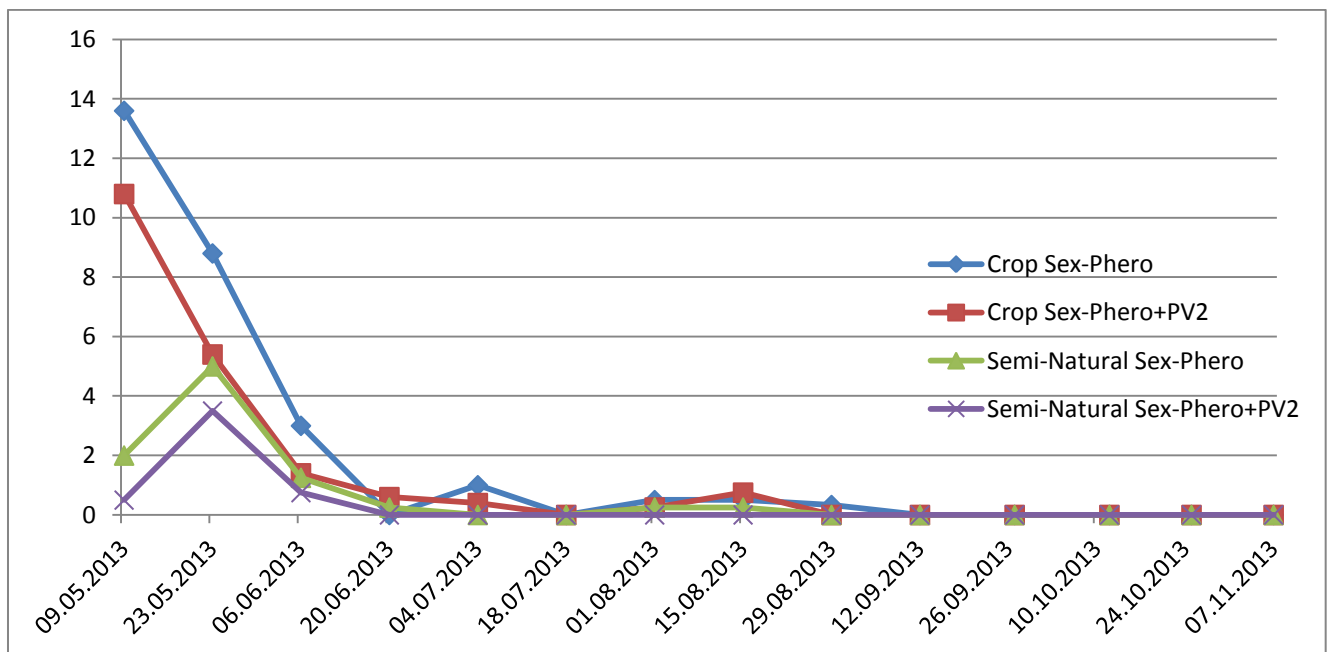


Fig. 13: Mean number of *L. rugulipennis* in sex-pheromone traps and kombi traps in the different habitats.

3.1.2. Trap and lure differences.

The collision traps and the traps baited with the PV2-lure alone caught only 17 *L. rugulipennis* through the season. The highest catches were seen in the traps baited with the *L. rugulipennis* sex pheromone alone (173), and the traps baited with the sex pheromone and PV2 caught second most (116). There was a significant difference between the traps baited with the sex pheromone alone and the traps baited with both the sex pheromone and the PV2 ($P=0.048$ $F_{1,15}=4.63$) using a general linear model.

3.1.3. Habitat differences

More *L. rugulipennis* were caught in the strawberry crops than in the semi-natural habitat. Using the same general linear model as above shows a significant difference in the catches between the two habitat types ($P < 0.001$ $F_{1,15} = 34.57$) with higher catches in the strawberry crops.

3.1.4. Nymph catches

The mean number of nymphs caught per site is shown in fig. 14. The peak of nymph catches was seen in the middle of July. No significant difference between the number of nymphs caught in the two habitats was seen ($P = 0.744$ $F_{1,8} = 0.11$).

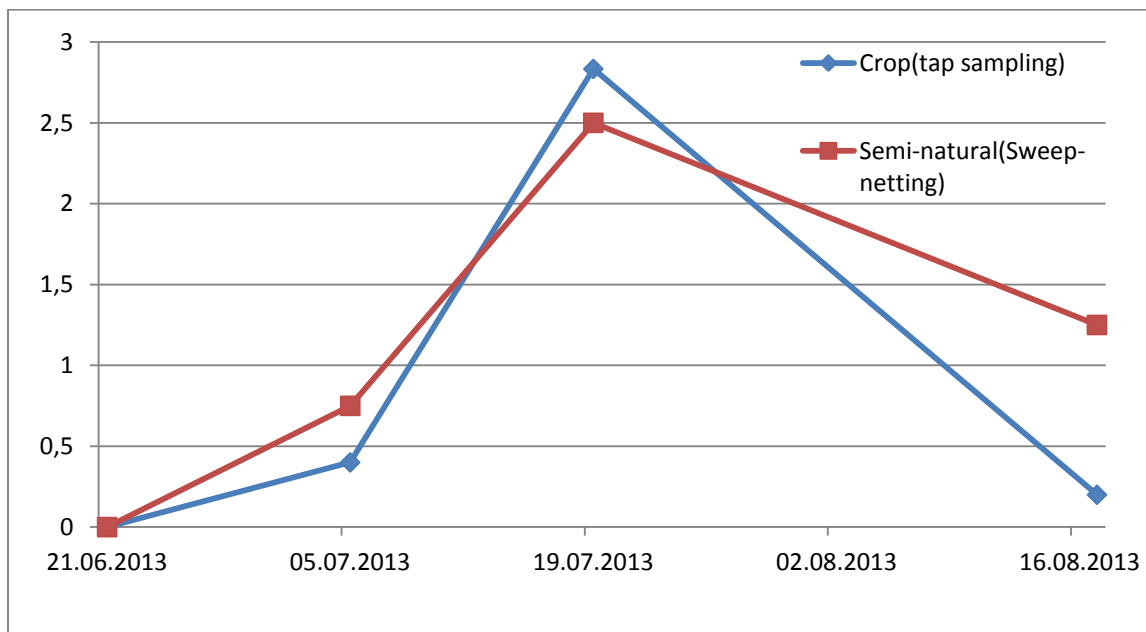


Fig. 14: Mean number of nymphs caught per site by tap sampling and sweep-netting.

3.1.5. Sex ratios

From trap deployment until June 20th 337 *L. rugulipennis* males and no females were caught in the funnel traps where the sex pheromone was present. In the same period 13 *L. rugulipennis* were caught in the collision trap with the sex ratio 10:3 males to females.

After the 20th of June 21 *L. rugulipennis* were caught in the sex pheromone baited traps (Sex ratio: 6:1) while no *L. rugulipennis* was caught in the collision traps.

Through the season the PV2 baited trap only caught 5 *L. rugulipennis* where 3 were males. Only 1 female was caught after the 20th of June.

3.1.6. Strawberry damage

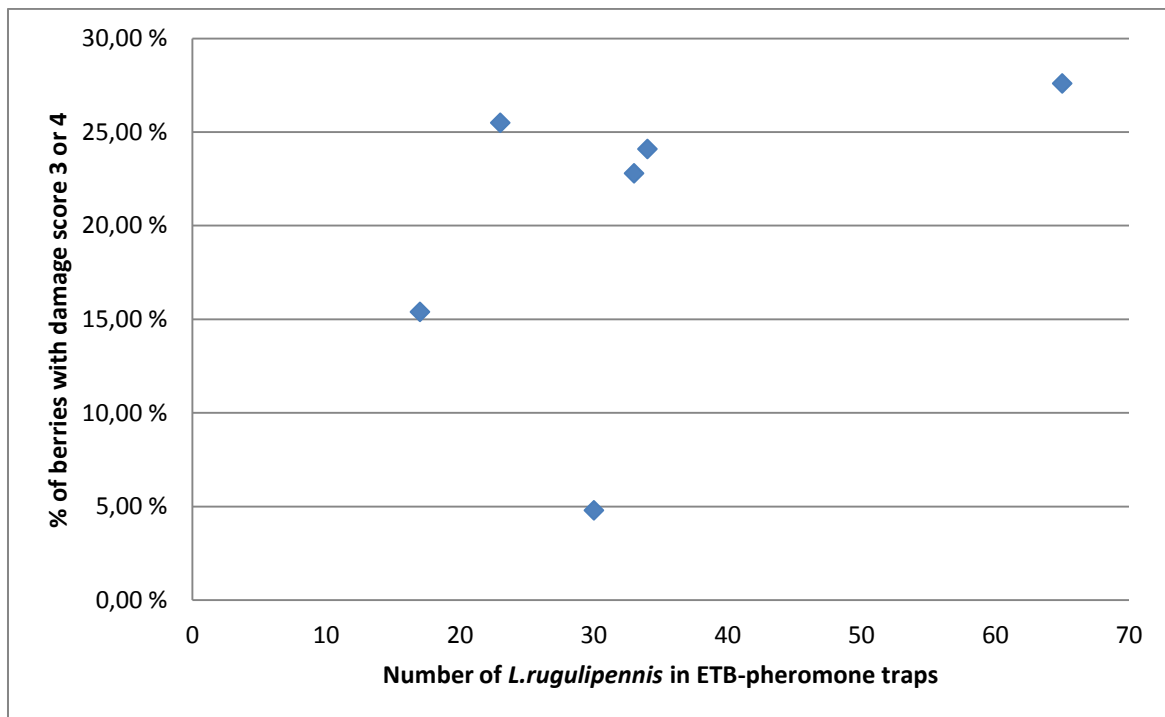


Fig. 15: Prosentage of berries with damage score 3 and 4 compared to number of *L. rugulipennis* caught in the *L. rugulipennis* sex pheromone trap.

The number of berries with damage score 3 or 4 varied from 4.8% to 27.6%. The highest number of damaged berries was seen at the “Huseby” site while the lowest number was seen at “Saxebøl Farm Strawberry 1”. The mean number of berries with score 3 and 4 were 20%.

3.1.7. *Anthonomus rubi* catches

A total of 68 *A. rubi* were caught in the traps during the season, of these only 3 were found in the semi-natural habitats. There was no significant difference in *A. rubi* catches between the traps baited with the *L. rugulipennis* sex pheromone and PV2, and the traps baited with PV2 alone. The catches in of two lure combinations in the crop are shown in fig.17

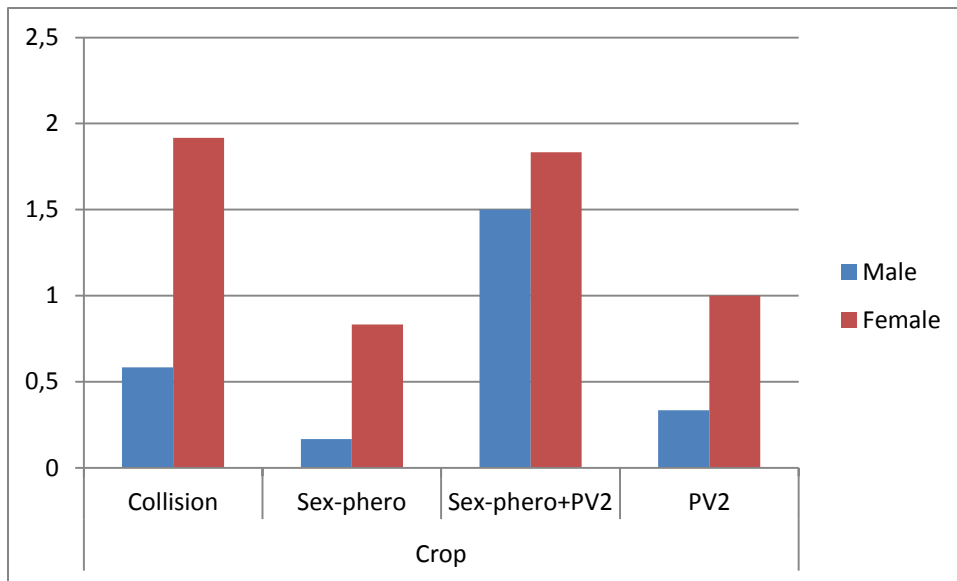


Fig.16: Mean number of *A. rubi* caught per trap in strawberry crops.

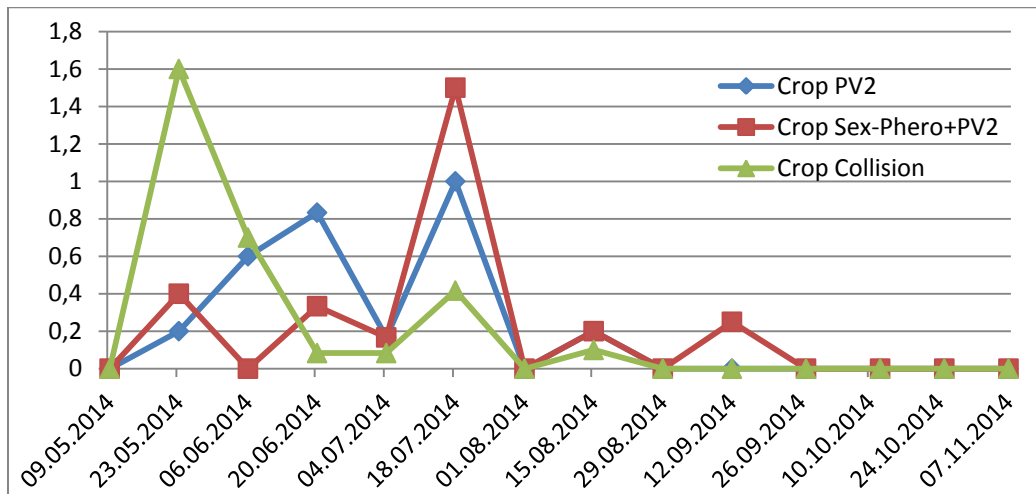


Fig.17: *A. rubi* catches per trap through the season in the Collision traps, the PV2 traps and the sex pheromone+PV2 traps in the crop.

There was a significant difference in *A. rubi* caught in the two habitat types tested, with more caught in the strawberry crops ($P=0.036$ $F_{1,17}=5.17$). No significant difference was seen between the traps baited with the PV2 alone and the traps with PV2 and the *L. rugulipennis* sex pheromone combined ($P=0,115$ $F_{1,17}=2,76$)

3.1.8. Pollinator catches

Bumblebees and bees were mainly caught by the neutral collision traps in both habitats, a total of 222 bumblebees were caught, of these 193(86.9%) were caught by the collision traps. 253 bees were caught in total, 249(98.4%) were caught by the collision traps. The mean number of bumblebees and bees caught per trap in both strawberry crops and the semi-natural habitat are shown in Fig.18.

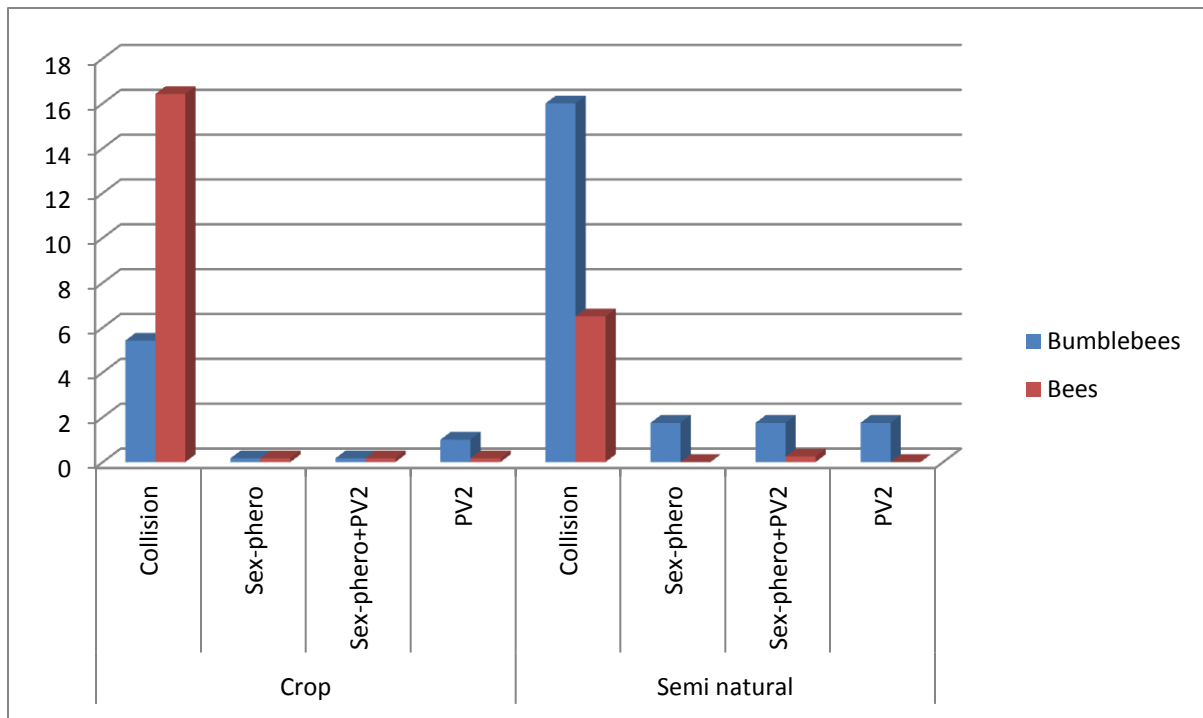


Fig.18: Mean number of *Bombus* and *Apis* caught per trap in the different habitats.

Most bumblebees were caught in the semi-natural habitat while the highest catches of bees were seen in the strawberry crops (Fig.18).

3.2. Behaviour study

3.2.1. Time spent on traps

The 42 *Mirids* observed on the trap spent on average four minutes on the trap, ranging from seven seconds to 22minutes and 22seconds.

3.2.2. Time of activity

As shown in fig.19 the *Lygus* were most active from early morning to mid day (06.00-12.00) and from late afternoon to late night (16.00-18.00) with the earliest sighting being at 06.53 and the last sighting before losing light was at 20.26.

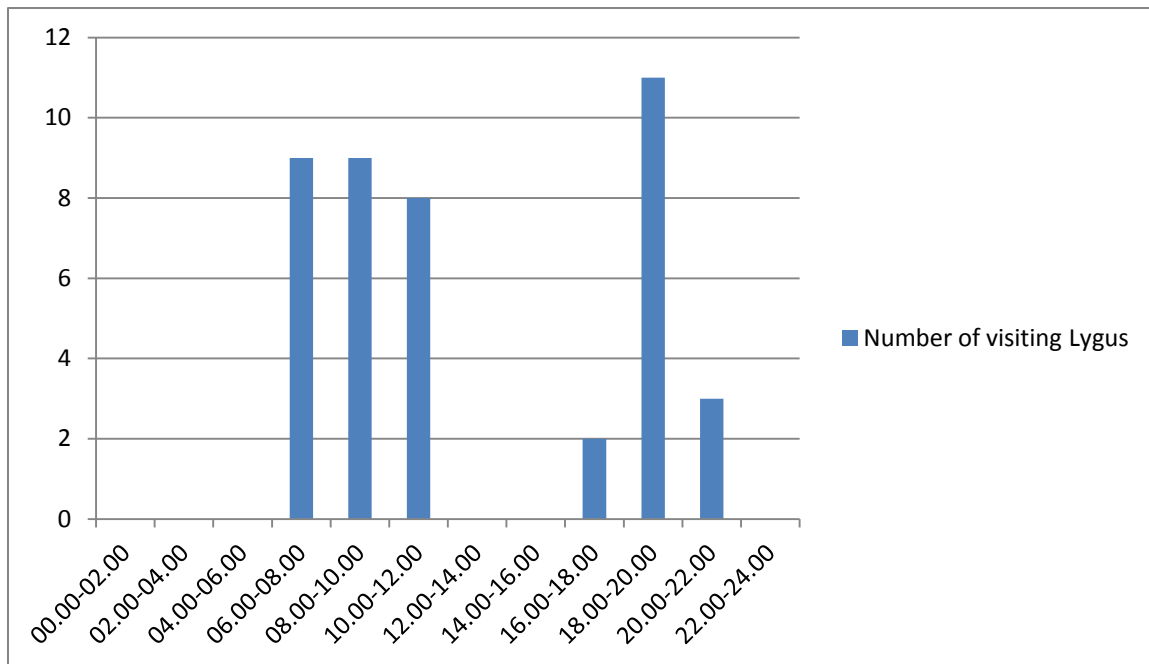


Fig.19: Number of visiting *Lygus* through the day.

3.2.3 Trap efficacy

The outcome of the trap behavior study is shown in fig.20. A total of 42 mirids visited the trap, but only three (7.1% \pm 7.7) were observed to fall into the trap. Fourteen (33.3%) were confirmed to fly away. The remaining 25 mirids observed on the trap walked out of the picture frame. If only counting the specimens with a known result, the percentage falling into the trap were 17.7% (3/17) and the percentage flying away 82.2% (Fig.20)

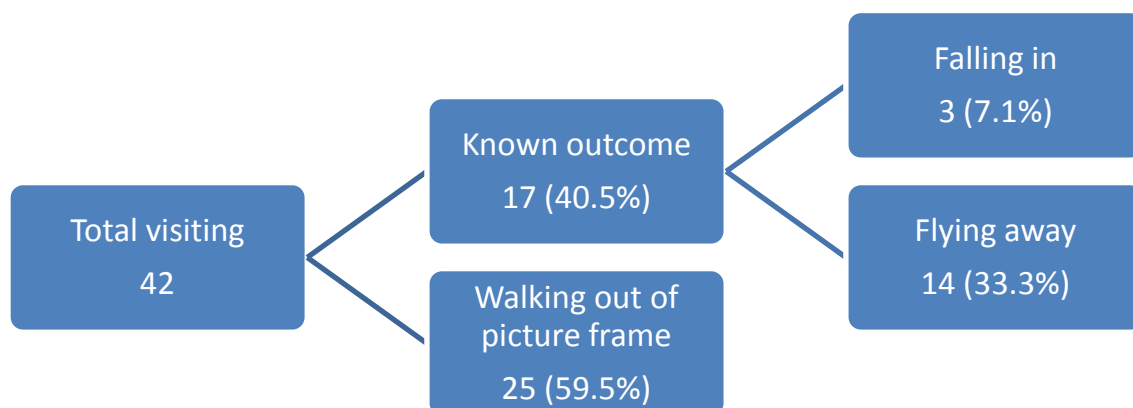


Fig.20: Outcome of behavior study

3.2.4. How they were caught

All three Mirids falling into the trap fell in by walking on the cross-vanes and slipping into the trap, no one flew and collided with the vanes before falling in.

3.3. Fluon trial

A total of 957 *L.rugulipennis* were caught in the trial, of these 56 females. 447 were caught in the control traps and 511 in the Fluon treated traps. The mean numbers of *L. rugulipennis* caught per trap are shown in fig.21.

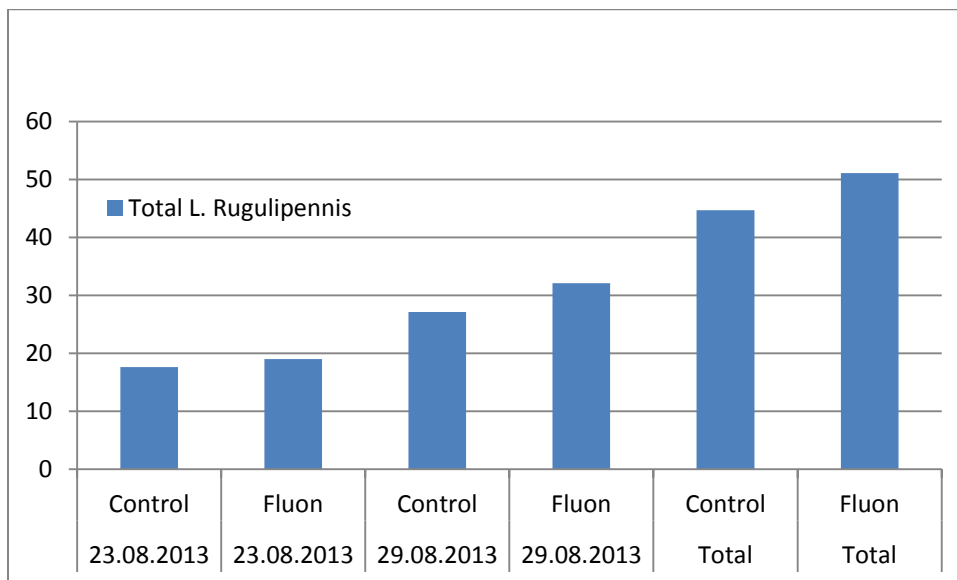


Fig.21: Mean number of *L.rugulipennis* caught per trap at the two collection dates and in total.

Both the paired t-test and the ANOVA ($P=0.241$ and $P=0.279$, respectively) showed no significant difference in catches between the control and fluon treated traps.

There was a significant change in catches in the fluon treated traps between the two collection dates with higher catches at the second collection date ($P=0.003$ $F_{1,19}=11.60$). However this was the tendency also in the control traps ($P=0.043$ $F_{1,19}=4.73$).

4. Discussion

The difference between *L. rugulipennis* numbers caught in traps baited with sex pheromone and collision traps or traps baited with PV2 only clearly show that the sex pheromone acts as an attractant to *L. rugulipennis* males.

4.1. Catch phenology

In Norway *L. rugulipennis* were almost exclusively caught early in the season (The end of April until the end of June) indicating that only males of the overwintered generation are attracted to the pheromone used. The pheromone blend mimics one used by *L. rugulipennis* females to attract males for mating. The assumption is therefore that only males who are ready for reproduction will be attracted to the pheromone blend. In Norway, and other countries, where *L. rugulipennis* is univoltine this will only be males of the overwintered generation. The lack of catches late in the season in Norway supports this assumption. In the UK where *L. rugulipennis* go through more than one generation a year, funnel traps baited with the sex pheromone is effective at least until the end of August (Fountain et al. 2014). This indicates that the *L. rugulipennis* sex pheromone is an effective lure for a larger part of the season in countries where the species go through more than one generation, and is further support of the assumption that only males ready for reproduction is attracted to the pheromone blend.

After the 20th of June, the catch in sex pheromone baited traps showed a sex ratio resembling that from the collision traps earlier in the season, however no *L. rugulipennis* was caught in the collision traps after June 20th. This might be because the overwintered *L. rugulipennis* die or migrate from the strawberry field after mating and oviposition. The lack of catches in the collision traps late in the season might also indicate that the new generation is not as active as the overwintered, or that there are no, or very few, *L. rugulipennis* of the new generation in the crops later in the season in Norway. However the presence of *Lygus* nymphs in the tap samples shows that *L. rugulipennis* are still present in the crops after the catches stop.

There was a significant difference between the traps with sex pheromone alone and the traps with sex pheromone together with PV2, the funnel traps baited with the *L. rugulipennis* sex pheromone alone catching most. This indicates that the presence of the PV2 lure has a negative effect on the *L. rugulipennis* catches at least early in the season. This means that the best way (of the methods tested) to control the *L. rugulipennis* populations is to deploy traps

baited with the *L. rugulipennis* sex pheromone without PV2, however PV2 is needed to catch *A. rubi*. Before the trial was started we hoped that PV2 would act as an attractant to the *L. rugulipennis* females, however there is no evidence that females were attracted to PV2.

4.2. Habitat difference in trap catches

There was a clear difference between the two habitats, with earlier and higher catches in the strawberry fields than in the semi-natural habitat. However the spraying in three of the fields might have suppressed an even later peak in the crop. In the remaining three fields, where the spraying was later, the peak catch was also later, or the decline slower (Figures showing early catches in each trap station are shown in appendix). The earlier and higher catches in the crop indicate that placing the funnel traps in the strawberry fields is preferable to placing them in the edges or in close proximity to the fields. However there is a chance that traps placed in strawberry will attract *L. rugulipennis* from surrounding habitats, depending on the attraction range of the lure, and cause a “spillover effect” (Switzer et al., 2009), as Ross and Daterman (1997) found with the douglas fir beetle (*Dendroctonus pseudotsugae*) where tree mortality caused by the beetle was concentrated around the trap sites. Moreover traps placed outside fields might attract *L. rugulipennis* from the crops, which might be positive even if they are not caught. Another possible consequence of placing traps in the strawberry crop is that they might be in the way of tractors and other equipment used in the fields. The growers on the farms used in this experiment did not have major problems with the funnel traps, however one of the farmers did experience some difficulties with the height of the traps in parts of the fields where the tracks between the strawberry rows were deep (Grower, Pers comm.).

4.3. Nymph catches in the light of the trapping results

In the tap sampling and in the sweep netting, the first *Lygus* nymphs appeared in early July, and peaked in mid July. The time between the catches of *L. rugulipennis* in the funnel traps stopped until the first nymphs were seen was 29 days. This is longer than the average egg stage of 21 days (Varis, 1971). However the egg development is greatly affected by temperature (Varis, 1972), and the tap- and net sampling were not frequent enough to precisely document the first emergence of nymphs. The appearance of nymphs after funnel catches had stopped support the assumption that the pheromone blend is not attractive to the new generation of *L. rugulipennis* in Norway.

There was no significant difference in number of nymphs in the two habitat types, indicating that the reproduction is just as high outside the strawberry crop as in the crop despite higher catches in the crops. However the methods used to collect nymphs were different in the two habitats and the effectiveness might differ.

4.4. The lack of females

Catching only males of a species have the potential to reduce the population, however to be an effective method of pest management the number of males caught has to be great enough and early enough to decrease the females mating rate (El-Sayed et. al. 2006). Traps using a sex pheromone to attract males of the sweet potato weevil (*Cylas formicarius*) greatly reduced the population, skewed the sex ratio, and reduced the mating ratio of females (Yasuda, 1995). However these traps caught males through the whole year, and numbers far higher than what were seen in with the *L. rugulipennis*. The sweet potato weevil is a multivoltine species (Mullen, 1981), and spends its whole life cycle in or near the host plant (Yasuda, 1995)

Attracting both sexes of the species would be preferable such as seen by Koczor et al. (2012) who found that both male and female *L. rugulipennis* were attracted to the plant odors phenylacetaldehyde and (E)-cinnamaldehyde, and concluded that traps baited with these odors could act as good monitor traps for *L. rugulipennis*.

4.5. Strawberry damage

The damage in this study varied from 4.8% to 27.6% which is well within the variation seen in other studies (Easterbrook, 2000; Labanowska, 2007). But still these numbers are lower than what was seen by Haaverstad (1979) who recorded that on average 41% of unripe strawberries and 42% of ripe fruits were “cat-faced”, however this study did not divide in different severity of damage. The percentage of undamaged fruits was still lower than in this study (64.5%). More than 20% damaged fruits is a serious loss of crop, however in practice the severity will depend on the threshold for selling damaged fruit. Consumers in Norway tend to tolerate fruits with slight damage when buying strawberries directly from the farmer (personal comment). In many other countries, or when selling to retailers, the tolerance of damaged fruits is lower and only undamaged fruits are salable.

4.6. *Anthonomus rubi* catches

The highest overall *A. rubi* catches were seen in the funnel traps baited with PV2. Early in the season the collision traps tended to catch more *A. rubi* than the PV2 baited traps, this might indicate that the *A. rubi* are not attracted to, or even repelled by PV2 early in the season, however the “catch surface” of the collision traps are much larger than on the funnel traps so as “neutral” traps they are expected to catch more. After the 6th of June the catches were highest in the funnel traps containing PV2 showing that PV2 acts as an attractant to the *A. rubi* later in the season. It does not seem like the *L. rugulipennis* sex pheromone has a negative effect on the *A. rubi* catches, as the highest number was caught in the traps baited with both lures. Only 3 *A. rubi* were caught in the semi-natural habitat, showing that the best placement of traps for mass trapping of the *A. rubi* most likely is in the strawberry fields rather than in surrounding habitats.

4.7. Pollinator catches

Of the total honey bees caught, only 1.6% was caught by funnel traps, for bumblebees it was 13.1%, whereof most of the latter (72%) were found in the semi natural habitat. This suggests that the lures and the color of the funnel traps do not act as attractants for bees. Funnel traps with green vanes, placed in strawberry crops are thus relatively safe for bees, and pollinator exclusion net covering the funnel opening is not needed. Such a net could potentially affect the *L. rugulipennis* trap efficiency negatively.

4.8. Trap efficacy (UK)

No *Lygus* was observed on the trap between 12.00 and 16.00, however two peaks in *Lygus* visits were seen, one between 06.00 and 12.00 and one between 16.00 and 21.00, suggesting that the *L. rugulipennis* males are not attracted to the sex pheromone blend during the day, but rather closer to the dusk and dawn. If the sex pheromone was active during the night was impossible to observe on the recordings, because of lack of light, however Šedivý and Honek (1983) found that the lowest flight activity of *L. rugulipennis* was between 01.00 and 07.00. The high activity seen at dusk are consistent with the results seen by Šedivý and Honek (1983) who found that the highest flight activity of the *L. rugulipennis* in the time between 18.00 and 01.00. Šedivý and Honek (1983) also found peaks in flight activity from 08.00-10.00 and from 14.00-15.00. This supports the findings that *L. rugulipennis* are most active

during the dusk and dawn, however the Šedivý and Honek (1983) experiment was done using a light trap, a completely different attractant than the sex pheromone blend.

A 95% confidence interval of probability requires the population (X) to be approximately normally distributed ($n \hat{p} (1-\hat{p}) \geq 5$). This condition was not fulfilled in this sample, therefore the confidence interval given is not strictly correct ($7.1\% \pm 7.7$), more observations of successful captures are needed to get more accurate information on trap efficacy. However, if these three days of observations were representative the trap efficacy is very low indeed.

Stewart (1968) found that the ovaries of overwintering female *L. rugulipennis* do not mature until spring, indicating that all mating of the overwintering generation appears in spring, making it possible to reduce the mating rate of females by catching males in spring. However with confirmed catches between 7% and 18% of the *Lygus* visiting, the trap design must be improved to ensure an effective trap for mass trapping of *L. rugulipennis*. El-Sayed et al. (2006) argue that one of the key aspects for making mass trapping an effective pest management method is that “the traps are efficient in catching and retaining attracted insects before they mate or oviposit”. Switzer et al. (2009) found that 69% of Japanese beetles (*Popillia japonica*) visiting funnel traps in a soybean field were eventually caught. However only 22% were caught on their initial approach, this number is still higher than the percentage caught in this experiment. The percentage of *L. rugulipennis* eventually caught is not known.

Wheeler (2001) report that the males of *L. hesperus* are able to mate up to 7 times, females do only need to mate once for producing viable eggs throughout the egg laying period, but are able to mate up to 3 times. Frati et al. (2008) reported that both sexes of *L. rugulipennis* are able to mate multiple times, and Varis (1971) found that the average number of eggs laid by a female *L. rugulipennis* was 72. This indicates that the catches of males have to be very high from early in the season to decrease the mating rate of females, and only catching between 7 and 18% of visiting males probably does not reduce the number of mated females significantly.

El-Sayed et al. (2006) concluded that mass trapping of the codling moth (*Cydia pomonella*) using a sex-pheromone to attract males was only effective in small and isolated populations, while in trials targeting high density population the mass trapping was unsuccessful. The success of mass trapping might be the case in this study as well, where the pheromone baited funnel traps might be more effective in small, isolated populations where the immigration of *L. rugulipennis* is limited.

The time spent on the trap (average of 4 min) should be sufficient to catch the visiting *L. rugulipennis*. The amount of time spent on the traps further support that the sex pheromone acts as a strong attractant to the *L. rugulipennis* males.

4.9. Fluon trial:

One obvious alternative is to make the surface of the trap in a more slippery material. Even though the number of *L. rugulipennis* caught in the fluon treated trap was higher than in the control treatment (510 and 447 respectively) the difference was not significant. Fluon does not make the traps sufficiently slippery to incorporate fluon in the trap design. Graham and Poland (2012) found that funnel traps where the cross vanes were coated with Fluon did catch more Cerambycid beetles than untreated traps. Even though there was no significant difference in catches of *L. rugulipennis* it was a tendency that fluon treated traps caught more than the control traps (8% higher on the first collection date and 18,5% on the second collection date). However in this trial the traps did not have the cross-vanes used in the traps in the Softpest project, and which are needed to catch the *A. rubi* efficiently.

There was a significant difference in the catches of *L. rugulipennis* in the fluon treated traps from the first collection date to the last with higher catches at the second collection date. However the same tendency was seen in the control treatment, if not as strong, indicating that this is caused by fluctuations in the *L. rugulipennis* population, or differences in the weather conditions. However the difference in catches in the control and fluon treated traps show the same tendency at both collection dates, indicating that the effect of the fluon does not wear off during this time. Graham and Poland (2012) also found that the effect of Fluon did not wear off in time, even though the traps in their trial was not exposed for weather over different time periods.

Getting a more slippery surface of the cross-vanes, on the funnel trap might be crucial to increase the catches of *L. rugulipennis*, since all of the Lygus confirmed to fall into the trap were walking on the cross-vanes and losing the grip.

To use transparent vanes instead of green ones might be another change of trap design that might be interesting to explore. Clear vanes might cause the *L. rugulipennis* flying towards the sex pheromone odor source to collide with the trap rather than land on the trap. However transparent could create problems with side catches of bees, as seen in the collision traps.

5. Conclusions

The *L. rugulipennis* sex pheromone blend does act as an effective attractant to the males of the species, however in countries where it goes through only one generation each year the sex pheromone lure are only effective in a small part of the season. To make this an effective mass trap the trap design has to be improved to ensure a higher proportion of visiting *L. rugulipennis* to get caught in the trap.

For both the *A. rubi* and the *L. rugulipennis* the highest catches were seen when the traps were placed in the strawberry crop, rather than in surrounding habitats.

The PV2 does not seem to attract females of *L. rugulipennis*, however it seems like it reduces the attraction of males to the sex pheromone blend, at least early in the season.

Traps baited with only the *L. rugulipennis* sex pheromone placed in the strawberry crops seem to be the most effective of the options tested for controlling the species and the sex pheromone did not reduce the number of *A. rubi* caught in the traps containing PV2.

Funnel traps with green cross vanes, and the tested lures do not attract bumblebees and honey bees, which mean that a bee exclusion net are not needed.

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7. Appendix

Figures A.1- A.10 shown below: Catches in the first part of the season for each of the 10 sites. The red cross indicates pesticide application. (Note differences in Y-axis)

