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# Exploring the Behavioural Responses of Strawberry Blossom Weevil and Two Other Non-Lepidopteran Pest Insects to Semiochemical Traps

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## PREFACE

First and foremost, I wish to thank Nina Trandem for being an amazing supervisor. Thank you for always leaving the door to your office open; for being creative and inspiring me to be the same; for feedback and discussions; for driving the jumble of me, traps, cans, and weevils everywhere; and not the least for good company. I also wish to thank my secondary supervisor, Atle Wibe. You have provided me with much helpful feedback, good discussions, brilliant ideas and much laughter.

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Softpest Multitrap is an international research project that was started in 2012. The aim was to develop efficient semiochemical traps to be used in mass trapping of *A. rubi*, *L. rugulipennis* and *B. tomentosus* in organic strawberry and raspberry fields.

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## ABSTRACT

Among the major pest insects of North-European berries are the strawberry blossom weevil *Anthonomus rubi* (Coleoptera: Curculionidae), the European tarnished plant bug *Lygus rugulipennis* (Hemiptera: Miridae), and the raspberry beetle *Byturus tomentosus* (Coleoptera: Byturidae). Softpest Multitrap is an organic research project aiming to decrease damage by these species, through optimal use of semiochemical traps for mass trapping. The overall objective of this thesis was to contribute with more knowledge of behavioural responses to cross-vane funnel bucket traps baited with species-specific lures, focusing on *A. rubi*.

The damage of *A. rubi* was assessed across several distances (0-12.30 m) from traps baited with lures (aggregation pheromones, plant volatile or both), in a field study. The objective was to see whether a “spillover” from the traps could be detected and whether this was affected by attractant used as lure. The results showed a significant increase in damage with distance from traps, thus dismissing the spillover hypothesis. Few weevils were caught, thus indicating the results were caused by too high release rate of the lures. The highest level of damage was around traps baited with aggregation pheromones alone and together with plant volatile, indicating a female preference for these lures.

The second objective was to learn how the trap work, how *A. rubi* behave on traps and how trap properties (lure and vane colour) and individual behaviour affects the capture process and rate. *A. rubi* behaviour on traps baited with aggregation pheromones, plant volatile or both, was documented by direct observations. Data from previous studies of *L. rugulipennis* and *B. tomentosus* was re-analysed. Estimated capture rate (i.e. percentage of individuals on trap that fell through the funnel and into the bucket) was low for all (2.7% for *A. rubi*). The probability of capture did not increase with time on trap for any of the three species. Most fell during the first five minutes on trap while others stayed for many more minutes without falling. This indicates individual differences in condition or the insects get increasingly used to the surface. Overwintered *A. rubi* were more restless than the new generation, which might affect the lower capture rates reported previously. Lure and vane colour had no effect on *A. rubi* on trap, suggesting their only role is attracting the individuals to the trap. The vanes were the area most occupied by all species which is an advantage for capture. Disadvantages discovered were too broad vanes with 20% stretching outside of the funnel opening, and the outer edges seemed to induce flight. Suggestions on how to improve trap design are narrower vanes, a more slippery vane surface, a slippery inner funnel, and a smoother junction between bucket and funnel part to ease the way for insects climbing from the ground.





# TABLE OF CONTENTS

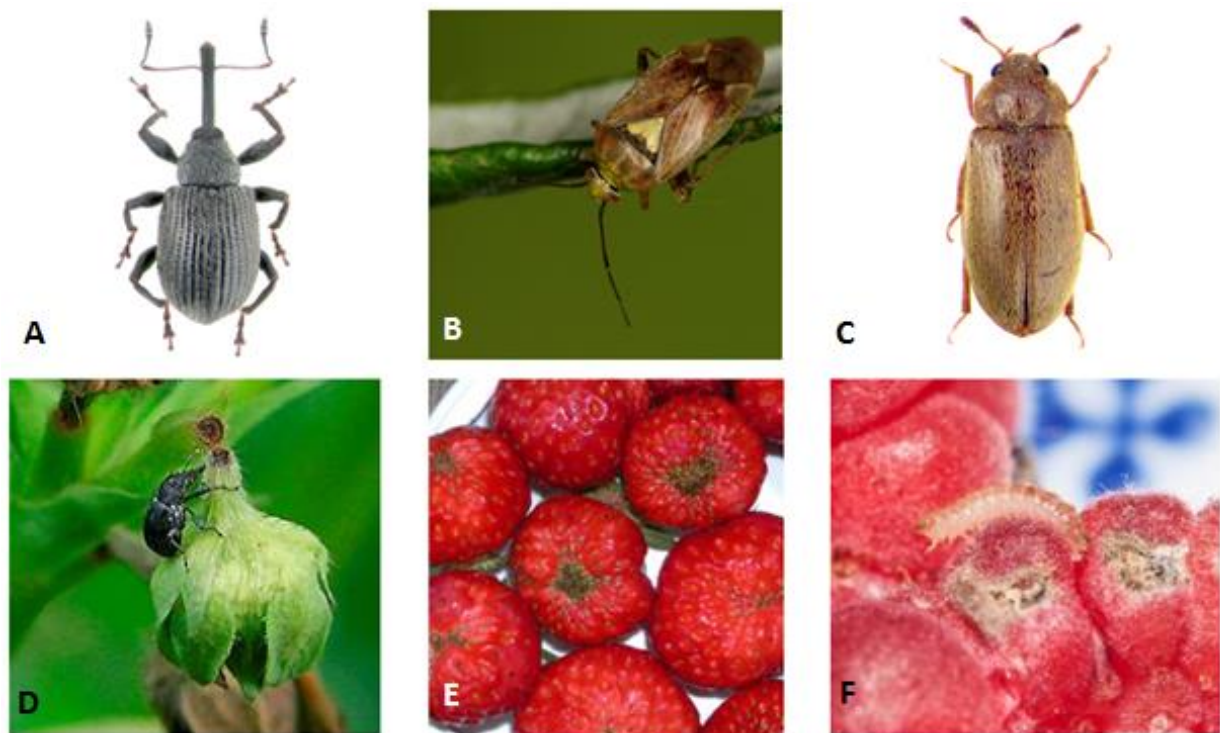
<b>1</b>	<b>INTRODUCTION.....</b>	<b>1</b>
1.1	LIFE CYCLE AND DAMAGE TO CROPS .....	2
1.2	BACKGROUND, CONTROL METHODS.....	- 4 -
1.3	SEMIOCHEMICALS AND THEIR USE IN PEST MANAGEMENT .....	- 5 -
1.4	THE SOFTPEST MULTITRAP PROJECT .....	- 6 -
1.4.1	<i>Semiochemicals used in Softpest Multitrap</i> .....	- 6 -
1.4.2	<i>Traps used in Softpest Multitrap</i> .....	- 8 -
1.5	MASS TRAPPING .....	- 9 -
1.6	OBJECTIVES .....	- 11 -
<b>2</b>	<b>MATERIALS AND METHODS .....</b>	<b>- 11 -</b>
2.1	TRAPS AND LURES .....	- 11 -
2.2	DAMAGE AROUND TRAPS TARGETING <i>A. RUBI</i> .....	- 13 -
2.3	BEHAVIOUR ON TRAP .....	- 14 -
2.3.1	<i>Observations of A. rubi on traps</i> .....	- 14 -
2.3.2	<i>Observations of L. rugulipennis on traps (re-analysis of data from earlier Softpest Multitrap study)</i> .....	- 18 -
2.3.3	<i>Observations of B. tomentosus on and around traps (re-examination of video footage)</i> .....	- 20 -
2.4	STATISTICS.....	- 22 -
2.4.1	<i>Damage around traps targeting A. rubi</i> .....	- 22 -
2.4.2	<i>Observations of behaviour</i> .....	- 22 -
<b>3</b>	<b>RESULTS .....</b>	<b>- 24 -</b>
3.1	DAMAGE AROUND TRAPS TARGETING <i>A. RUBI</i> .....	- 24 -
3.2	BEHAVIOUR ON TRAP .....	- 25 -
3.2.1	<i>Overview of behaviour on the presumed most efficient trap for each target species</i> .....	- 25 -
3.2.3	<i>Effect of lure and vane colour on A. rubi behaviour</i> .....	- 29 -
3.2.4	<i>Effect of sex, trap treatment, days without food and behaviour on status at the end of the observation</i> .....	- 31 -
<b>4</b>	<b>DISCUSSION .....</b>	<b>- 33 -</b>
4.1	DAMAGE AROUND TRAPS TARGETING <i>A. RUBI</i> .....	- 33 -
4.1.1	<i>Spillover effect?</i> .....	- 33 -
4.1.2	<i>Differences between the two fields</i> .....	- 34 -
4.1.3	<i>Low capture</i> .....	- 35 -
4.2	OBSERVATIONS OF BEHAVIOUR ON TRAP.....	- 35 -



4.2.1	<i>Capture</i> .....	- 35 -
4.2.2	<i>Behaviour on trap</i> .....	- 38 -
4.2.3	<i>Factors affecting behaviour and capture</i> .....	- 39 -
<b>5</b>	<b>CONCLUSIONS</b> .....	<b>- 42 -</b>
5.1	DAMAGE AROUND TRAPS TARGETING <i>A. RUBI</i> .....	- 42 -
5.2	OBSERVATION STUDIES.....	- 42 -
5.2.1	<i>Improvements in trap design</i> .....	- 44 -
<b>6</b>	<b>REFERENCES</b> .....	<b>- 46 -</b>

## 1 INTRODUCTION

Among the major pest insects of North-European berries are the strawberry blossom weevil [*Anthonomus rubi* Herbst (Coleoptera: Curculionidae)], the European tarnished plant bug [*Lygus rugulipennis* Popp. (Hemiptera: Miridae)], and the raspberry beetle [*Byturus tomentosus* De Geer (Coleoptera: Byturidae)]. Measures to control these insects are crucial for farmers of strawberry and raspberry crops. Farmers of organic berry crops, which lack effective control measures, can experience 65% direct loss of strawberries because of *A. rubi* (Trandem et al. 2004) and 50% unmarketable strawberries because of *L. rugulipennis* (Jay et al. 2004). In organic raspberry crops *B. tomentosus* might render over 50% of the berries unmarketable (Arus et al. 2013). To manage these species, their life cycle and biology should be considered.



**Figure 1** Three major pest species of strawberry and raspberry. Upper row: Adults A) *Anthonomus rubi* (www.zin.ru). B) *Lygus rugulipennis* (photographer: L. Skipper, from www.miridae.dk). C) *Byturus tomentosus* (www.zin.ru). Bottom row: Pictures of damage by the three species in the same order (photographer of D and E: N. Trandem, and of F: E. Fløistad. All from leksikon.bioforsk.no).

## 1.1 Life cycle and damage to crops

*A. rubi* is an important pest because it stops the development of berries as part of its life cycle. The overwintered adults emerge in April, during the pre-flowering stage of strawberry plants (Stenseth 1970; Kovanci et al. 2005). They feed on buds and foliage, and begin ovipositing by the start of flowering (Stenseth 1970). The female deposits an egg into the flower bud before she makes punctures in the stalk (Jary 1931). The puncturing stops the development of the bud; it dries up and often falls off the plant to the ground (Jary 1931). Usually one egg is deposited per bud, but the females may cut off a whole cluster of buds for one single egg or even cut buds without ovipositing (Jary 1932; Aasen et al. 2004). Easterbrook et al. (2003) found in a lab study that mean fecundity was 157.6 eggs which means that even at low densities, potential crop losses are great. The development from egg to adult happens inside the withering bud. The time of development is dependent on the temperature, and range from 18.2 days at 25°C to 95.5 days at 10°C (Easterbrook et al. 2003). In Norway, the new generation adults appears in July (Stenseth 1970). They feed on foliage of different types of plants before overwintering (Stenseth 1970), but does not cause important damage on the crops until the next spring (Jary 1932; Alford 1984). Other than living in cultivated strawberry and raspberry fields, *A. rubi* also feeds on and develops in other plants, mostly of the rose family (Jary 1931; 1932).

*L. rugulipennis* is a highly polyphagous species and a pest of many cultivated plants (Holopainen & Varis 1991). Among these, it is a serious pest species of strawberries (Jay et al. 2004), while there is normally no need for control in raspberries (Gordon et al. 1997). Adults and nymphs both feed on flowers and developing fruits making the berries malformed and lighter of weight than undamaged berries (Taksdal & Sørum 1971; Easterbrook 1997). Eggs of *L. rugulipennis* are deposited singly or in groups into the plant tissue (Varis 1972). Easterbrook et al. (2003) found that mean fecundity at 20°C was 75.4 eggs, ranging from 23 to 179. This gives potential for a high damage rate, and Varis (1972) showed that one adult *L. rugulipennis* injured 24.2 sugar beet plants in average. Females were feeding more frequently than males, and did more damage (Varis 1972). In the UK it is considered a serious strawberry pest especially on late season crops because they flower when the mirid is abundant (Easterbrook 1997).

**Table 1** Overview of three major pest species of strawberry and raspberry in North-Europe: *Anthonomus rubi*, *Lygus rugulipennis* and *Byturus tomentosus*. Their taxonomy is shown, some aspects of their life cycle, distribution as pest species in Europe, and lastly, whether they are pests of strawberry, raspberry or both.

	<b>Common name</b>	<b>Taxonomy</b>	<b>Voltinism (in the study areas of this thesis)</b>	<b>Overwintering</b>	<b>Distribution as pest species in Europe</b>	<b>Strawberry or raspberry pest</b>
<i><b>Anthonomus rubi</b></i>	Strawberry blossom weevil	Coleoptera: Curculionidae	Uni in Norway (Stenseth 1970)	One winter as adults, in litter along boundaries and in berry crop fields (Jary 1931)	Locally important in European countries, especially in southern regions (Gordon et al. 1997; Aasen & Trandem 2006)	Both (Stenseth 1970; Alford 1984)
<i><b>Lygus rugulipennis</b></i>	European tarnished bug	Hemiptera: Miridae	Bio in the UK (Easterbrook 1997)	One winter as adults, in the substrate of coniferous forests or in strawberry fields (Varis 1972; Easterbrook 1997)	Widespread in northern and central parts of Europe, and sometimes also southern Europe (Holopainen & Varis 1991; Gordon et al. 1997)	Strawberry (Taksdal & Sørum 1971; Gordon et al. 1997)
<i><b>Byturus tomentosus</b></i>	Raspberry beetle	Coleoptera: Byturidae	Semi in Norway (Stenseth 1974)	Two winters; one as larvae, the second as adults, in the berry crop soil (Stenseth 1974; Gordon et al. 1997)	Widespread throughout Europe (Gordon et al. 1997)	Raspberry (Gordon et al. 1997)

Number of *L. rugulipennis* generations a year differs between locations. In Finland and Norway one generation a season is recorded (Varis 1972), while Easterbrook (1997) found nymphs occurring two times a season in the UK. The UK study showed that the adults emerged from overwintering in Mid-March and were found afterwards mainly on weed hosts. On these hosts, the first generation of nymphs occurred and developed into adults. Development time from egg to adult is dependent on temperature and range from 83.4 days at 15°C to 28.8 days at 25°C (Easterbrook et al. 2003). Many of the first generation adults migrated into the strawberry fields in late June or early July. At this time, late season crops are still in flowering. Also increasing numbers of another generation nymphs were found during July-August in the strawberry crops. Adults occurred here until early November.

*B. tomentosus* does not damage strawberry, but the larvae develops in raspberries. By their presence and feeding, the contaminated fruits become unmarketable. In addition, the tunneling allows fungal pathogens to enter the berry and quicken the rotting process (Woodford et al. 2001). The *B. tomentosus* adults emerge from the soil in spring and shortly afterwards begin ascending the plants where they feed on buds and flowers before and during mating (Stenseth 1974; Willmer et al. 1996). The eggs are deposited in open flowers, usually one egg per flower (Taylor & Gordon 1975; Willmer et al. 1998). Taylor and Gordon (1975) found that the eggs began to hatch during the green-fruit stage. Soon after, the larvae started tunneling from the basal fruit drupelets into the berries. They fed on the inside of the drupelets and on the receptacle. It was observed that one larva might contaminate more than one fruit, as larvae entered new fruits by leaving and entering via the receptacles. When the larvae were fully fed, they left the berries to overwinter and pupate in the soil. The development from larvae to adult usually takes two years in Norway (Stenseth 1974).

## 1.2 Background, control methods

To manage *A. rubi*, *L. rugulipennis* and *B. tomentosus* no effective control measures exist for organic farmers, while conventional farmers use insecticides. Insecticides do reduce the damage (Jay et al. 2004; Aasen & Trandem 2006; Baroffio et al. 2011), but they have become a public concern because of residues left in the fruit and the negative effects they may have on the ecosystem, especially on the pollinators and natural predators of pest species (James & Xu 2012; Bolli et al. 2013). That pests will develop resistance against the pesticides is also a main concern. In the case of *A. rubi*, scientists and farmers have reported that the insecticides used

in Norway are insufficient against the weevils (Aasen & Trandem 2006). In several parts of Norway, *A. rubi* are found resistant against pyrethroids (Trandem et al. 2006). Consequently, berry producers are warned against using more than absolutely needed of this pesticide to slow down further selection of the mutation. This leaves few options to prevent *A. rubi* from building up its population. Mortality factors such as weather conditions may keep the population from increasing (Łabanowska 2004; Wibe et al. 2014a), but the most certain control measure is to establish new fields away from the old ones after three years of harvest (Aasen & Trandem 2006; Haslestad & Trandem 2011). To repeat this every third year however, can be costly, unpractical and challenging for the farmer due to lack of appropriate land. Therefore, considering resistance in target species, restrictions on insecticides and public concern, both conventional and organic farmers are in need of new control measures. Methods based on deliberate manipulation of behaviour of the pest insects can be such an alternative measure.

### 1.3 Semiochemicals and their use in pest management

The behaviour of insects are triggered by various types of stimuli (Foster & Harris 1997; El-Sayed et al. 2006; Witzgall et al. 2010). Several pest management programs exploit the use of semiochemicals to reduce or prevent damage (El-Sayed et al. 2006; Witzgall et al. 2010). Semiochemicals are chemical compounds used in intra- or interspecific communication. Intraspecific semiochemicals are called pheromones, produced by the insects to e.g. attract a mate. Interspecific semiochemicals is in the present study limited to those produced by plants, i.e. plant volatiles. Many insects use plant volatiles to locate resources, such as feeding and oviposition sites, and mates may also be encountered on the plant.

The most common semiochemical-based approaches are mate disruption and mass trapping (El-Sayed et al. 2006). In mate disruption, attractants are used to delay, reduce or prevent fertilization, by misdirecting or disorienting the individuals searching for a mate (Foster & Harris 1997; El-Sayed et al. 2006; Witzgall et al. 2010). In mass trapping, attractants are used to lure and trap enough individuals to reduce the damage or even eradicate the pest population (El-Sayed et al. 2006). One of the benefits of using semiochemicals in pest management is that they are unlikely to target other species than the pest. Not all chemical compounds stimulate a response in a species, making the identification of attractive chemical compounds an extensive study (Bichão et al. 2005a; Bichão et al. 2005b).

## 1.4 The Softpest Multitrap project

Successful mass trapping of *A. rubi*, *L. rugulipennis* and *B. tomentosus* was the goal of a three-year research project called Softpest Multitrap (Core Organic II 2012). This project aimed to develop a semiochemical mass trapping system that would effectively and without insecticides, lower the damage of the three pest species (Core Organic II 2012). To do this, trials in six different countries in central and northern Europe were carried out 2012-2014 (Core Organic II 2012; Wibe et al. 2014b). Upon the project's beginning, semiochemicals attracting the three target species had already been discovered and synthesized independently: Aggregation pheromones and a plant volatile for *A. rubi*, a sex pheromone for *L. rugulipennis*, and a flower volatile for *B. tomentosus*.

### 1.4.1 Semiochemicals used in Softpest Multitrap

#### *A. rubi* aggregation pheromone

Pheromones produced by males to attract a mate are often called aggregation pheromones because individuals of both sexes respond (Landolt 1997). This system in which females are searching for males and not the other way around, is an uncommon one, as pointed out by Landolt (1997). Aggregation pheromones are perhaps best known from bark beetles (Coleoptera: Byturidae) where the pheromones attract members of both sexes to aggregate and exhaust the tree's defences before finding a mate and reproducing (see e.g. Raffa et al. (1993) for review). Even so, Alcock (1982) among others (Raffa et al. 1993; Landolt 1997) argue that the main function of an aggregation pheromone is to attract a mate and reproduce. Their arguments include that the male usually signals from plants important to the female as oviposition or feeding sites, and consequently, female responders get access to both host and males; male responders might be opportunists responding to enhance their own signal or to intercept a mate of their own. Curiously, most of the known male aggregation pheromones are found within the Coleoptera, in contrast to sex pheromones which is mostly found in the Lepidoptera (Landolt 1997). In accordance with this, compounds of the *A. rubi* male aggregation pheromone were identified: lavandulol and grandlure I and II (Innocenzi et al. 2001). Grandlure I and II refer to the components of the aggregation pheromone of the boll weevil *Anthonomus grandis* (Tumlinson et al. 1969; Innocenzi et al. 2001). The compounds were collected from *A. rubi* males while they fed on *Fragaria ananassa* plants (Innocenzi et al. 2001). In the same study, it was shown that they were attractive for overwintered *A. rubi* of

both sexes. Moreover, later studies show that also the new generation weevils are attracted (Cross et al. 2006a).

#### *Combination of A. rubi aggregation pheromone and plant volatile*

In many cases, a relation exists between host plant volatiles and aggregation pheromones that makes the pheromone more attractive to the receiver (Reddy & Guerrero 2004; Witzgall et al. 2010). The relation might be that chemicals are synergized from host plant chemicals (Raffa et al. 1993), or the plant volatiles stimulate the production of pheromones (Jaffé et al. 1993), or simply the presence of the plant volatiles makes the signal more attractive (Landolt 1997). The possible benefits of this relation are many, the most apparent being that the responder will save energy by finding both mates and host at the source (Landolt 1997). Landolt et al. (1992) suggested the possibility of females being able to distinguish between good or bad hosts through such signals, or that host plant volatiles alone were less detectable to females than male pheromones. The last was also suggested by Roseland et al. (1990) in a study where females of the red sunflower seed weevils *Smicronyx fulvus* did not locate flowers suited for oviposition unless it was infested with males. In *A. rubi*, a relation between host plant volatiles and aggregation pheromones was supported when males failed to produce the aggregation compounds in the absence of *F. ananassa* (Innocenzi et al. 2001). In addition, several *F. ananassa* volatiles were induced by *A. rubi* feeding (Innocenzi et al. 2001; Bichão et al. 2005a; 2005b). One volatile in particular, germacrene-D, increased more than ten-fold either as a response to feeding damage or because of production by the weevils (Innocenzi et al. 2001). In light of this and previous studies on other species, Innocenzi et al. (2001) suggested that induced strawberry plant volatiles might have an effect on the release or production of *A. rubi* aggregation pheromones. However, when Cross et al. (2006b) and later Wibe et al. (2014a) tested the effect of germacrene-D together with the *A. rubi* pheromones, the plant volatile did not have a significant effect on the capture. Wibe et al. (2014a) however, did find synergism between the aggregation pheromone and a second tested volatile, 1,4-dimethoxybenzene, which was dominant in previously studied wild strawberry *Fragaria vesca*. In their study, over twice as many weevils were caught in traps baited with both pheromone and the “new” volatile, as in traps baited with only pheromone.

#### *L. rugulipennis sex pheromone*

Sex pheromones are produced to attract individuals of the opposite sex. Usually the female produce the pheromone. Compounds of the female *L. rugulipennis* sex pheromone were



discovered and synthesized by Innocenzi et al. (2004). However, the attraction to the synthetic pheromone in field was not demonstrated until later (Fountain et al. 2014). Innocenzi et al. (2005) found that the synthetic blends had higher success in glass microcapillary tubes than in sachets and polyethylene vials, probably because the emission from these more closely resembled the emission from females. These lures however, were only attractive for a few days in field during which the blends were released in variable rates (Innocenzi et al. 2005; Fountain et al. 2014). Subsequently, the three compounds were put into plastic pipette tips by Fountain et al. (2014) who also did an extensive study on its release and attraction. They found that this new dispenser released constant blends in the same rate as produced by females and over at least 30 days. Hence, the release of attractants had to coincide with the time of calling in females, which was also found to be the time when females were most attractive to males (Fountain et al. 2008; Fountain et al. 2014). Most importantly, it was revealed that traps with these lures were more efficient in capturing *L. rugulipennis* males than traps baited with virgin females (Fountain et al. 2014).

#### *B. tomentosus*, flower volatile

The raspberry flowers are important to *B. tomentosus* as feeding, mating and oviposition sites (Stenseth 1974; Taylor & Gordon 1975; Willmer et al. 1998). Birch et al. (1996) suggested that raspberry flower volatiles play a part in *B. tomentosus* discrimination between hosts. Several flower volatiles attractive for both sexes of the *B. tomentosus*, were identified as outlined by Birch et al. (1995; 1996). Of these volatiles, especially one compound of raspberry flowers called “Compound B”, was an effective attractant in field (Woodford et al. 2003). However, in trapping studies where this volatile is used, raspberry flowers still seem to be more attractive than the semiochemical resulting in two catch periods a season: before and after flowering (Woodford et al. 2003; Ekeland 2005; Baroffio & Mittaz 2008).

#### 1.4.2 Traps used in Softpest Multitrap

The traps used in Softpest Multitrap were funnel traps with cross-vanes. As with the semiochemicals used in this project, these traps were developed and used in studies independently, before the beginning of the project.

In the early studies on mass trapping of the three species, variations of a white plastic board with sticky surface were used (Innocenzi et al. 2001; Woodford et al. 2003; Innocenzi et al.

2005). When compared to other trap designs, this type captured the highest number of *A. rubi* (Cross et al. 2006b). However, on two occasions there was a pattern of higher *A. rubi* damage in the trap area than in areas without traps suggesting the weevils were attracted but the traps were unable to catch them (Lethmayer et al. 2004; Cross et al. 2006a). The traps were also highly impractical to handle because of the glue.

To catch *B. tomentosus*, a trap which used visual cues as well as olfactory, was developed by inserting non-UV reflective cross-vanes (not sticky) into the funnel trap (Birch et al. 2008). Variations of this trap was later used in Softpest Multitrap to capture *A. rubi* and *B. tomentosus* in raspberry crops (Wibe et al. 2014b). In strawberry crops a similar combination, but with green vanes, were decided on to capture *A. rubi* and *L. rugulipennis* (Fountain et al. 2014; Wibe et al. 2014b; Fountain et al. 2015). This trap had a higher capture rate than the sticky traps, did not become saturated, was more practical to handle, and the green vanes lowered the by-catch of beneficial insects (Trandem et al. 2008; Fountain et al. 2014; Wibe et al. 2014b; Fountain et al. 2015).

## 1.5 Mass trapping

The success of mass trapping varies with different studies (El-Sayed et al. 2006; Witzgall et al. 2010). The key to short term success is traps to capture enough insects before they damage the crops or reproduce (El-Sayed et al. 2006). El-Sayed et al. (2006) listed five requirements for this, where four concerned the lures and traps: 1) the lures must be perceived by the species and 2) be more attractive than natural resources. Also, 3) the traps must catch and hold the attracted insects before they mate or oviposit, and 4) be effective for as long as the adults emerge and mate. The potential for long term success with mass trapping might be measured by a decrease in trap catches or a decrease in damage over time (El-Sayed et al. 2006). Mass trapping of *B. tomentosus* with funnel traps with white cross-vanes baited with flower volatile has shown potential as damage to crops decreased during three years of trapping (Baroffio et al. 2011). However, not in areas where immigration from wild raspberry was a possibility. Thus the pest species biology and population dynamics is a factor to be considered to fulfil El-Sayed et al. (2006) requirements.

Many other factors need to be investigated as well to evaluate the potential of mass trapping: trap density and interference, effective attraction range, trap design, and features of the lures

(such as dosage and dispenser) to mention some (El-Sayed et al. 2006). Prior to Softpest Multitrap, Cross et al. (2006a) investigated trap interference and attraction range of sticky traps with aggregation pheromone. While trap interference could not be found, they noted that there seemed to be higher *A. rubi* damage around the traps. By comparing a test field to a control field Lethmayer et al. (2004) noticed the same. Similar results were obtained by Switzer et al. (2009) in a study of mass trapping of Japanese beetles *Popillia japonica*. Upon closer inspection, they found that the females did not land on the traps baited with sex pheromone, but on plants near the traps. Most likely, the females were not attracted to the precise location of the lure, but to the general area of it. The females then attracted males, making an aggregation around the trap. As a result, the damage increased in the plants around the trap without increasing the capture. Switzer et al. (2009) called this a “spillover effect”. A spillover from traps targeting *A. rubi* might still lower the capture in spite of additional attractants and new traps. (However, since only the female *A. rubi* damage the plants, this effect would be somewhat more difficult to discern.)

Lures and trap design should be adapted by the behavioural responses of the pest species towards the traps. The trap must be easy for the species to locate and enter but difficult to leave (Phillips & Wyatt 1992; Foster & Muggleston 1993). As trap design even affects the plume of the lure (Lewis & Macaulay 1976), relatively small details might affect the success or failure of a trap (Phillips & Wyatt 1992). Most studies where trap designs are compared the capture is compared. Capture is the goal, so naturally this is important. However, details about why one trap is more successful than another might be overlooked (Phillips & Wyatt 1992). In their paper, Phillips and Wyatt (1992) argued how direct observations will show the exact details of how the pests contact the trap and are captured (or not captured). For instance, there is some indirect evidence as to how *A. rubi* contacts the traps (Innocenzi et al. 2001; Cross et al. 2006b), but it is not known how they behave on the trap or how they fall. Suggestions are that the insects are arrested by the lure and walk on the vanes until they fall from exhaustion; they let themselves fall when startled; or they slip when trying to land. Storberget (2014) found that *L. rugulipennis* lose grip when walking on the vanes and fall. *B. tomentosus* may also lose the grip and fall, but they also slip when trying to land (Blagogie 2010). Without observations it is impossible to know if *A. rubi* fall in a similar way. Direct observations might also help elucidate how to further improve the traps used in Softpest Multitrap.

## 1.6 Objectives

The main purpose of this study was to reveal the efficiency of the cross-vane funnel traps used in the Softpest Multitrap project. Objectives that concerned the behaviour of *A. rubi*, and also *L. rugulipennis* and *B. tomentosus* were decided on, as these would add more detailed knowledge to the project:

The first objective was to see whether a spillover effect or other patterns could be detected around baited traps targeting *A. rubi*, and see whether this was affected by which attractant was used as lure. Accordingly, traps baited with different lures were deployed in strawberry fields and plant damage was recorded across different distances from the traps.

The second objective was to learn how *A. rubi* behave on traps and to see whether trap properties (lure and vane colour) and individual behaviour affects the capture process and rate. Thus, behavioural indicators for successful captures would be identified and an understanding of how the trap works be achieved. To achieve this, the behaviour of individuals that fell into the trap was compared with that of the individuals that escaped. Older studies of *L. rugulipennis* and *B. tomentosus* were added to see whether similarities in the patterns between the three species targeted could be detected.

## 2 MATERIALS AND METHODS

### 2.1 Traps and lures

In all studies, funnel traps with cross-vanes were used (Figure 2). These traps consisted of a funnel leading into a bucket, cross-vanes above the funnel, and a cap on top to protect the traps from rain. To kill the insects falling into the traps, buckets were half filled with water mixed with 0.1% Triton to break surface tension. In the field study on *A. rubi* damage, traps with green cross vanes were used. In the observational studies, white non-UV reflective cross-vanes were used for *B. tomentosus*, green for *L. rugulipennis*, and both vane colours were tested on *A. rubi*. The traps for *A. rubi* and *L. rugulipennis* stood on the ground fastened with steel wires so they would not fall. The trap for *B. tomentosus* hung from a pole one meter over ground.



**Figure 2** Pictures of the cross vane traps used in the different studies. A) Picture of the type of cross vane trap used in observations of *Anthonomus rubi* and *Lygus rugulipennis* behaviour. The cross vanes were of either green or white non-UV reflective plastic. B) Picture of the trap used in observations of *Byturus tomentosus*, with white non-UV reflective vanes.

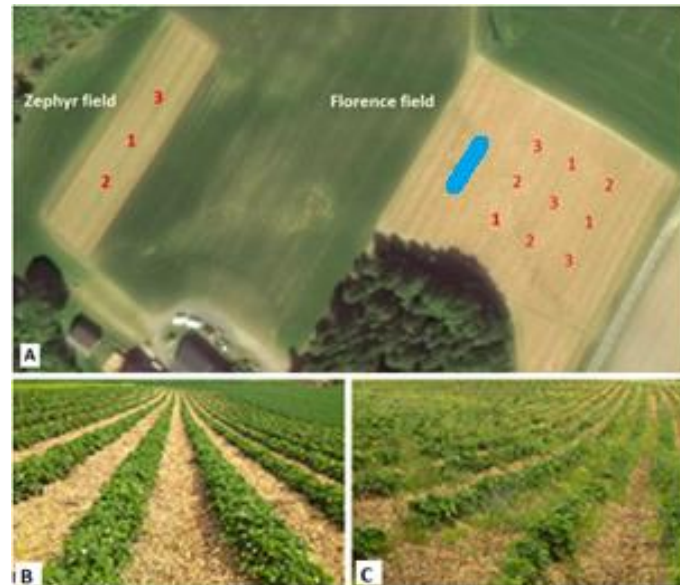
The semiochemicals used in the *A. rubi* studies were in sachets that were put into the basket underneath the cap of the trap (Figure 2A). The *L. rugulipennis* lure was also put into the basket, but this lure was in the form of a pipette as described by Fountain et al. (2014). The lure for *B. tomentosus* was in a sachet that was fixed by tape underneath the cap of the trap (Figure 2B). An overview of the lures is shown in Table 2.

**Table 2** An overview of the semiochemicals used as attractions for three different insect species. In the *Anthonomus rubi* studies, a plant volatile and aggregation pheromones were tested separately and in combination.

Species	Semiochemical used as attractant (short names in parentheses)	References
<i>A. rubi</i>	Plant volatile (PV): 1,4-dimethoxybenzene	Wibe et al. (2014a)
	Aggregation pheromone (AgPh): Grandlure I, Grandlure II and lavandulol	Innocenzi et al. (2001)
	Blend of the plant volatile and aggregation pheromone (PV+AgPh)	Wibe et al. (2014a)
<i>L. rugulipennis</i>	Female sex pheromone: Hexyl butyrate, (E)-2- hexenyl butyrate and (E)-4-Oxo-2-hexenal	Innocenzi et al. (2004); Fountain et al. (2014)
<i>B. tomentosus</i>	Flower volatile: “Compound B” (identity not published)	Birch et al. (1996); Woodford et al. (2003)

## 2.2 Damage around traps targeting *A. rubi*

In the studies of effective trap range two co-located strawberry fields were used (Figure 3). The first field, cultivar ‘Florence’, was planted in 2012 (“Flor field”) and the other field, cultivar ‘Zephyr’, in 2011 (“Zeph field”). The Flor field was about 110x110m, while the Zeph field was about 110x22m. The fields were located in Ås, South Eastern part of Norway (59°39'53"N 10°41'11"E).



**Figure 3** The field experiment where *A. rubi* damage was assessed around baited traps. A) Positions of *Anthonomus rubi* traps in two located strawberry fields. “Zephyr” field to the left and “Florence” field to the right. Numbers in red are traps baited with aggregation pheromone (1), plant volatile (2), and both (3). The blue area marks a patch without strawberry plants. B) Picture from the Zephyr field taken 03.06.2014. C) Picture from the Florence field taken 01.07.2014.

In total, 12 traps were set up: nine in the Flor field and three in the Zeph field (Figure 3A). (The Zeph field was included because of a weedy patch without strawberry plants in the middle of a row in the Flor field.) The traps were baited with PV, AgPh or PV+AgPh. On the establishment of the study sites (13<sup>th</sup> May 2014), only a few flower buds were present in the Flor field (BBCH=57), while in the Zeph field plants were in flower (BBCH=61).

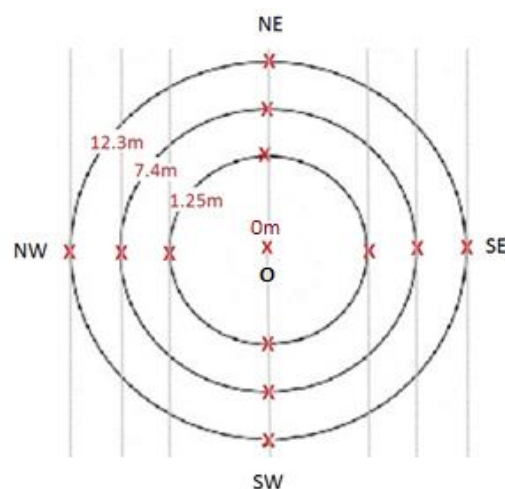
The number of *A. rubi* caught in the traps were recorded 21 and 37 days after the start of the experiment in both fields. At the same time, cut buds per plant were counted in one plant at three distances (1.25m, 7.4m and 12.3m) in four directions from every trap, and in the plant closest (0m) to the trap (Figure 4). In the Zeph field, the counts at 12.3m to South East and North West were excluded since the field was too narrow. On the last inspection date, cut buds were counted in the Flor field only (in the Zeph field most undamaged buds were then developed into flowers or berries. In the rest of the text “final count” is therefore the one count in the Zeph field and the second count in the Flor field).

On random plants in both Flor field (18 plants) and Zeph field (10 plants) the total number of flowers (flowers, buds, stalks from cut off buds, and berries) was recorded at the first inspection date. This was to allow estimation of percent damage.

## 2.3 Behaviour on trap

### 2.3.1 Observations of *A. rubi* on traps

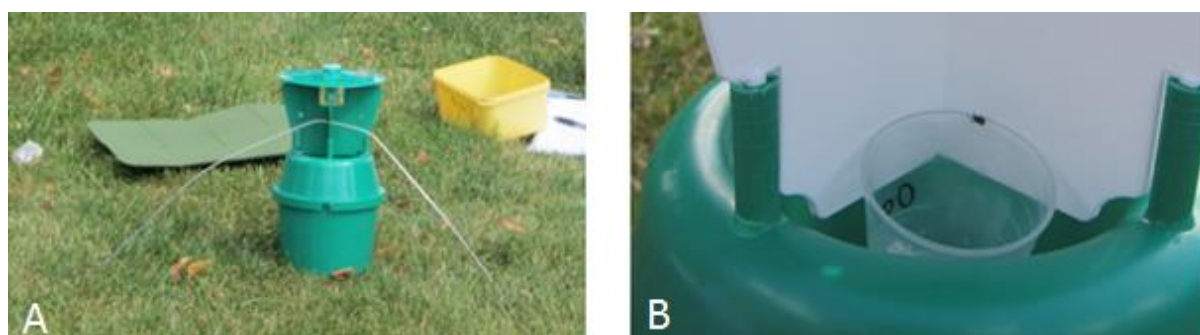
Overwintered weevils were tap sampled in a conventional strawberry field in Ås, close to the Flor and Zeph fields (59°39'28"N 10°41'04"E) at 14<sup>th</sup> May 2014, and kept together in a terrarium with access to food and water until used in the behavioural study. Tap sampling yielded few new generation weevils. Instead, severed buds were collected from a later bearing strawberry field in Frogn (59°41'25"N 10°41'55"E) 9<sup>th</sup> July and kept in boxes at room temperature to rear adult weevils. Before the observations most of the new generation adults were kept in boxes with access to water, but not food (for 0-7 days). Some of the weevils were put in a terrarium with access to both food and water.



**Figure 4** Spatial arrangement of cut bud counts made around each of the 12 traps in the field experiment. Cut buds were counted at four distances in four directions. Each red ‘X’ marks a plant where cut buds were counted. (The X in the middle = the plant closest to the trap). Numbers in red are distances in meters from the trap. Letters in black are the direction from the trap (O = trap, NE= North East, SE = South East, NW = North West, SW = South West).



The observations were conducted during two periods in 2014: one in May with overwintered generation weevils, and one in July-August with new generation weevils. The observations were carried out over 12 days in total, three for the overwintered and nine for the new generation. Both generations were observed at Ås university campus (59°39'57"N 10°46'25"E) on traps placed in the middle of a lawn (about 40x50m) (Figure 5A). One to three hours before the observations started, adults of *A. rubi* were put singly in small containers together with moist paper. The weevils would not fly to the trap, so the containers were placed into the funnel one at a time to let the weevil walk onto the trap by itself (Figure 5B). The observations started at 1300 hours and lasted until the area was in the shade, at 1900-2100 depending on the month (the weevils were slow to leave their containers after this time).



**Figure 5** Direct observation of *Anthonomus rubi* behaviour on cross-vane funnel traps. A) One of the traps used in the observations, fastened with steel wire in the middle of a lawn. B) The weevils were kept in containers that were placed on the traps. The observations began as the weevil left the container and walked onto the trap.

After having walked onto the trap, overwintered generation weevils were observed for maximum five minutes, and each weevil's behaviour sequence was recorded by hand onto a drawing of the trap. New generation weevils were observed for 15 minutes and the time spent in different parts of the trap was recorded. Later behaviour sequences of both generations were coded into behavioural categories used for analysis (listed in Table 3). A note of the behaviour sequence of the new generation weevils was taken at five minutes to allow some comparison of categories between the two generations, and to see if the behaviour changed after five minutes. The status of the weevil at the end of the observation time was either 'stayed on trap for 5min', 'stayed on trap for 15min' (for new generation weevils only), 'escaped/attempted escape' or 'fell into bucket'. It was also noted from which part of the trap (c.f. Figure 2) the weevils fell into bucket or escaped/attempted escape. Each weevil was only observed once.



**Table 3** Overview of behavioural categories used to describe and analyse the behaviour of *Anthonomus rubi* on traps, during the observation time (maximum 5 minutes for overwintered generation, 15 minutes for new generation). Data was collected by direct observations. The last three categories (in capital letters) are three mutually exclusive categories used to describe the status of each weevil at the end of the observation time.

<b>Generation</b>	<b>Behavioural category</b>	<b>Description of behaviour</b>	<b>Behaviour measured as</b>
<b>Over-wintered</b>	Approached lure area	Went to lure area one or two times	Occurrence of behaviour (1/0)
	Showed lure oriented behaviour	Went to lure area three or more times	Occurrence of behaviour (1/0)
<b>New</b>	Time in lure area	Was in lure area for a measured time	Residence time (seconds)
	Approached lure area	Was in lure area for less than 10 seconds in total	Occurrence of behaviour (1/0)
	Showed lure oriented behaviour	Was in lure area for 10 seconds in total or more	Occurrence of behaviour (1/0)
	Time on vanes	Was on vanes for a measured time	Residence time (seconds)
<b>Both</b>	Time on trap	Stayed on any part of the trap (except upper cap surface and bucket) for a measured time	Residence time (seconds)
	Went to lure area	Went to lure area at some point during the observation	Occurrence of behaviour (1/0)
	Stayed on vanes only	Did not leave the vanes for the whole time on trap	Occurrence of behaviour (1/0)
	Went to outer vane edge	Went to the outer edge of the vanes at some point during observation	Occurrence of behaviour (1/0)
	Went to other area than vanes	Went to funnel or underside of cap at some point during the observation	Occurrence of behaviour (1/0)
	STAYED FOR 5/15 MINUTES	Stayed on any part of the trap (except upper cap surface and bucket) for the whole observation period	Occurrence of behaviour (1/0)
	ESCAPED/ ATTEMPTED ESCAPE	Flew or prepared to fly (opened its wings/climbed on upper cap surface/climbed out on the steel wire/walked out on the funnel edge)	Occurrence of behaviour (1/0)
	FELL INTO BUCKET	Was captured by falling through the funnel and into the bucket	Occurrence of behaviour (1/0)

Different trap treatments (combination of vane colour and lure) were tested in a random new order every day, always one at a time. Each treatment was used on 1-2 weevils in a row, before a new treatment was used on the next 1-2 weevils. This was to avoid biased results from day, time of day and other unknown covariates. A total of 186 *A. rubi* were observed (Table 4). On the first day of observing the new generation weevils, the observations (N=9) lasted max five minutes. This gave a higher sample size for the five minutes studies than the 15 minutes studies in the new generation.

**Table 4** Number of overwintered and new *Anthonomus rubi* observed on traps with different lures and vane colour. The different lures tested were plant volatile (PV) and aggregation pheromone (AgPh). A control without lure was also tested. For the overwintered generation weevils, cross vane traps with green vanes were used for all the lures. For the new generation both white and green vanes were tested. N = number of weevils observed.

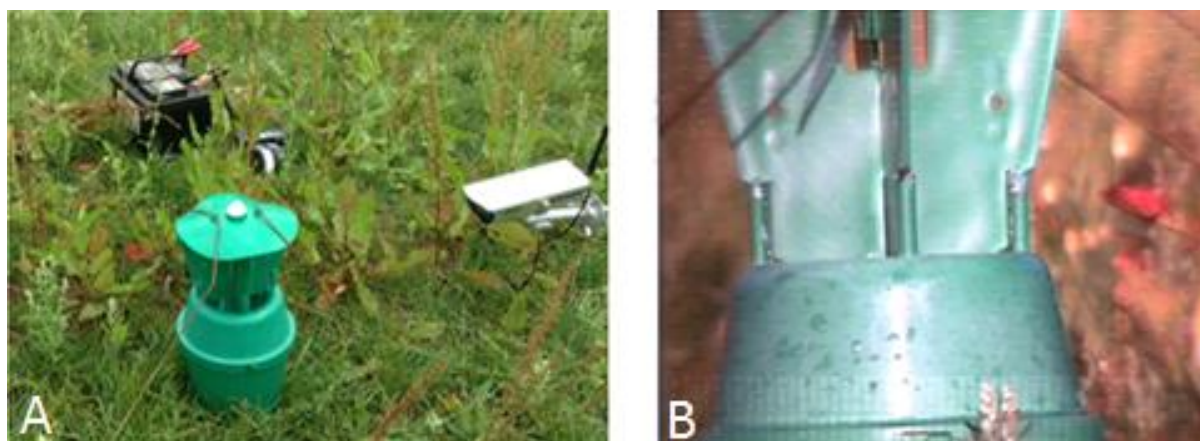
Generation	Vane colour	Lure	N
<b>Overwintered</b> - Each weevil was observed for max five minutes - Total N=75	Green	None (control)	11
		Pv+AgPh	21
		PV alone	20
		AgPh alone	23
<b>New</b> - Each weevil was observed for max 15 minutes (except for N in parenthesis which were observed for max five minutes) - Total N=100 (+9)	Green	None (control)	18 (+2)
		Pv+AgPh	17 (+1)
		PV alone	19 (+2)
		AgPh alone	18 (+2)
	White	None (control)	14 (+2)
		Pv+AgPh	14

To prevent contamination from lures between the different treatments, one trap was used for each lure, and the traps were kept in separate bags. The lures stayed in the baskets for the whole study and disposable plastic bags were used when handling the baskets. The baskets with the lures were put in separate bags in the fridge when they were not being used.

Weevils that had not escaped during the observation were euthanized by alcohol for further examination. The thorn on the males' intermediary coxae was used to sex the weevils (Innocenzi et al. 2002).

### 2.3.2 Observations of *L. rugulipennis* on traps (re-analysis of data from earlier Softpest Multitrap study)

A *L. rugulipennis* study was conducted by Fountain (unpublished material) and Storberget (2014) as part of project Softpest Multitrap, in Kent, UK, at the grounds of East Malling Research (51°17'20''N 0°27'18''E). A trap baited with sex pheromone was set up with steel wires in a set aside area of annual weeds containing *Chenopodium album* and *Matricaria spp.* with a camera to record the behaviour of *L. rugulipennis* attracted (Figure 6). Recordings were taken during five days in late July-middle of August 2013, in between 0600 and 2030 hrs. After this hour, they could not examine the recordings because of the dark. The recordings were examined at 5x speed on a computer monitor to document the behaviour sequences of the mirids (e.g. Walking up vane>flies to funnel edge and walks around>flies to vanes>flies to other side of trap) and duration of each encounter. An encounter begun as a mirid came into the picture frame (on trap) and lasted until it disappeared from view. For the present study, these behaviour sequences were recoded into behavioural categories similar to those used for *A. rubi* (section 2.3.1) to see if these could predict whether the mirid escaped or fell into bucket (Table 5). As Storberget (2014) excluded the data he did not collect himself for his thesis, behavioural sequences from both him and Fountain (unpublished material) were used in the present study.



**Figure 6** Setup for recording of behaviour of *Lygus rugulipennis* attracted to a cross vane trap lured with sex pheromone. A) The trap and camera as they were placed in the weed field. B) Screen shot of the video tape from which the behaviours were documented. Both pictures from M. Fountain, East Malling Research, UK (unpublished material).

**Table 5** Overview of the behavioural categories used to describe and re-analyse the behaviour of *Lygus rugulipennis* during each encounter on trap. Behaviour sequences were collected from previous studies and coded into categories for the present study. The last three behavioural categories (in capital letters) are three mutually exclusive categories used to describe the mirid's status at the end of the encounter.

<b>Behavioural category</b>	<b>Description of behaviour</b>	<b>Measured by</b>
Minimum time spent on the trap	Stayed on any visible part of the trap for a measured time	Residence time (seconds)
Went to lure area	Went to lure area at some point during the encounter	Occurrence of behaviour (1/0)
Went to vanes	Went to the vanes at some point during the encounter	Occurrence of behaviour (1/0)
Stayed on vanes only	Did not leave the vanes during the whole encounter	Occurrence of behaviour (1/0)
Went to funnel/bucket	Went to the funnel or bucket at some point during the encounter	Occurrence of behaviour (1/0)
Stayed on funnel/bucket only	Did not leave the funnel/bucket area during the whole encounter	Occurrence of behaviour (1/0)
Went to cap	Went to the cap at some point during the encounter	Occurrence of behaviour (1/0)
ESCAPED	Flew away from the trap	Occurrence of behaviour (1/0)
FELL INTO BUCKET	Was captured by falling through the funnel and into the bucket	Occurrence of behaviour (1/0)
UNKNOWN	Disappeared out of the picture frame	Occurrence of behaviour (1/0)

### 2.3.3 Observations of *B. tomentosus* on and around traps (re-examination of video footage)

Blagodie (2010) recorded by video *B. tomentosus* behaviour on and around traps in May-June 2009 in Ås, South-Eastern part of Norway (59°40'10''N 10°46'56''E). The observations were carried out during four days between 1400 and 1900 hrs. This was in a seminatural forest patch dominated by wild raspberry. A cross vane trap with a *B. tomentosus* lure hung on a pole one meter above ground (Figure 7). Individuals of *B. tomentosus* less than one meter from the trap were filmed with a handheld camera (Sony Handycam). During video examination, each beetle coming into view was treated as an encounter, lasting until the beetle disappeared from view. The behavioural sequence and residence time in different parts of the trap was documented (time on vanes, bucket, cap, other areas around the trap), also the time spent flying, and how the encounter was ended (the beetle escaped or fell into bucket). For the present re-examination study, new behaviour categories were added ('went to lure area' and 'stayed only on trap') (Table 6). Some categories were modified ('flew' no longer included flying before landing on trap or after escape, and 'went to the cap' only included the upper surface). It was also noted how the beetles made contact with the trap, which area of the trap they contacted first, and from which part of the trap they fell into bucket or escaped (c.f. Figure 2).



**Figure 7** Behaviour of attracted *B. tomentosus* was recorded by filming a cross vane trap baited with flower volatile. For the present study, the videos were re-examined. These two screen shots are from videotapes recorded by Godfred Blagodie.

**Table 6** Overview of the behavioural categories used to describe and analyse the behaviour of *Byturus tomentosus* during each encounter on trap. The behavioural sequences were described by re-examination of film recordings. The last two behavioural categories (in capital letters) are mutually exclusive categories used to describe the beetle's status at the end of the encounter.

<b>Behavioural category</b>	<b>Description</b>	<b>Measured by</b>
Visited the trap	Made contact with the trap	Occurrence of behaviour (1/0)
Time spent on trap	Stayed on any part of the trap for a measured time	Residence time (seconds)
Went to lure area	Went to the lure area at some point during the encounter	Occurrence of behaviour (1/0)
Time on vanes	Stayed on vanes for a measured time	Residence time (seconds)
Went to vanes	Went to the vanes at some point during the encounter	Occurrence of behaviour (1/0)
Stayed on vanes only	Did not leave the vanes during the whole encounter	Occurrence of behaviour (1/0)
Went to outer vane edge	Went to the outer edge of the vanes at some point during the encounter	Occurrence of behaviour (1/0)
Went to bucket	Went to the bucket at some point during the encounter	Occurrence of behaviour (1/0)
Went to cap	Went to the upper surface of the cap at some point during the encounter	Occurrence of behaviour (1/0)
Flew	Flew from the trap and landed back on the trap at some point during the encounter	Occurrence of behaviour (1/0)
ESCAPED	Flew further than one meter away from the trap	Occurrence of behaviour (1/0)
FELL INTO BUCKET	Was captured by falling through the funnel and into the bucket	Occurrence of behaviour (1/0)

## 2.4 Statistics

The statistical software Minitab 17.2 was used for all analysis. To test for correlation between variables, Pearson correlation was used for numerical data and Fisher's exact test for binominal data (variables that gave a Pearson correlation coefficient of above 0.9, or  $P$  less than 0.05 in Fisher's exact test, were considered to be highly correlated). Variables that were highly correlated were not put in the same model. Backward elimination was used to find the best model. The significance level for all tests was 0.05, but all  $P$ -values of less than 0.1 are mentioned.

### 2.4.1 Damage around traps targeting *A. rubi*

Number of cut buds in the final counts were analysed using general linear model (GLM) with the explanatory factors lure (PV/AgPh/Pv+AgPh), direction (NE/NW/SE/SW/O), and field (Flor/Zeph), and trap distance (0.00-12.30m) as a covariate. Also, the interaction between lure and distance was included in the model. Standard error (SE) from the mean ( $\bar{X}$ ) was calculated for cut buds per plant, and total number of flowers, buds, stalks, and berries per plant.

### 2.4.2 Observations of behaviour

Binary logistic regression (BLR) was used to model the occurrence of behaviours (yes/no). GLM was used to model residence time. The overwintered generation and the new generation *A. rubi* were tested in separate models.

*Overview of behaviour on the presumed most efficient trap for each target species:*

For all three species, some descriptive statistics were run first, estimating the trapping/escape rate and the most important characteristics of the behaviour observed. Only the lures that were presumed to be the most efficient were used, therefore only the results from the trap baited with PV+AgPh was used for *A. rubi* (Wibe et al. 2014a). (Green vanes were used to allow some comparison between the overwintered and new generation).

*Effects of lure and vane colour:*

To see if trap treatment could explain the *A. rubi* behaviour, lure and vane colour were set as explanatory variables. For the overwintered generation of *A. rubi*, 'lure' (PV/AgPh/Pv+AgPh), was explanatory variable. For the new generation, 'vane colour' (green/white) was added and

interactions between the two explanatory variables. The response variables analysed were (cf. Table 3): ‘stayed for 5min’ (yes/no), ‘stayed for 15min’ (yes/no, only for new generation), ‘escaped/attempted escape’ (yes/no), ‘fell into bucket’ (yes/no), ‘showed lure oriented behaviour’ (yes/no, only for overwintered generation), ‘stayed in lure area’ (seconds, only for new generation), ‘stayed on vanes only’ (yes/no).

*Adding other factors (behaviour, sex, days without food) to explain the status at the end of the observation:*

For *A. rubi* (cf. Table 3 for a description of behavioural categories), factors tested in both generations were: ‘lure’ (PV/AgPh/Pv+AgPh), ‘stayed only on vanes’ (yes/no) and ‘sex’ (male/female). In the model for overwintered generation, the predictor ‘showed lure oriented behaviour’ (yes/no) was added, while ‘stayed in lure area’ (seconds) and ‘days without food’ (0-7) was added to the new generation model. *A. rubi* behavioural categories used as responses were: ‘stayed for 5min’ (yes/no), ‘stayed for 15min’ (yes/no, only for new generation), ‘escaped or attempted escape’ (yes/no), ‘fell into bucket’ (yes/no).

For *L. rugulipennis* (cf. Table 5 for a description of behavioural categories), explanatory variables were: ‘went to the lure area’ (yes/no), ‘went to the bucket’ (yes/no), ‘went to the cap’ (yes/no), ‘went to the vanes’ (yes/no) and ‘stayed only on vanes’ (yes/no). The behaviours on vanes (‘went to the vanes’ and ‘stayed only on vanes’) were two measurements of the same behaviour, so these were run in two separate models for each response. Responses (excluding the ‘unknown’ category) were: ‘escaped’ (yes/no), ‘fell into bucket’ (yes/no) or ‘time spent on the trap’ (seconds).

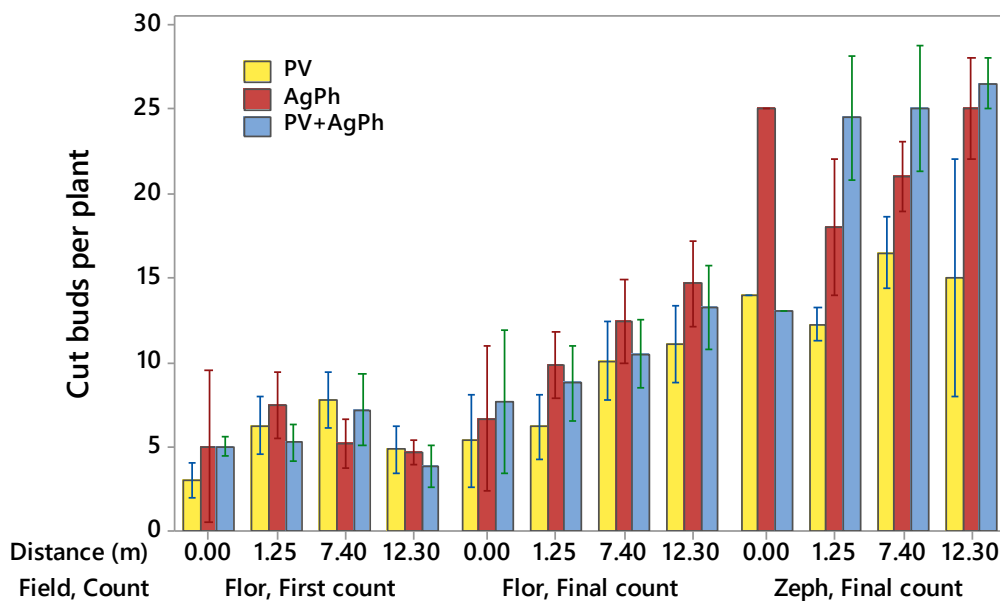
For *B. tomentosus* analysis (cf. Table 6 for a description of behavioural categories), explanatory variables were: ‘went to lure area’ (yes/no), ‘went to the bucket’ (yes/no), ‘went to the cap’ (yes/no), ‘flew’ (yes/no), ‘stayed on vanes’ (seconds) and ‘stayed only on vanes’ (yes/no). The two measurements of behaviour on vanes were run in two separate models for each response (as for *L. rugulipennis*). Responses were: ‘escaped’ (yes/no), ‘fell into bucket’ (yes/no) and ‘time spent on the trap’ (seconds).



### 3 RESULTS

#### 3.1 Damage around traps targeting *A. rubi*

The variables ‘field’, ‘distance from trap’ and ‘lure’ had significant effect on number of cut buds/plant in the final count (field:  $F_{1,148}=47.47$ ,  $p<0.001$ , distance:  $F_{1,148}=12.14$ ,  $P=0.001$  and lure:  $F_{2,147}=4.28$ ,  $P=0.016$ ). The average level of damage was highest in the Zeph field (Figure 8). By the final count the mean plant damage (mean number of cut buds a plant/mean number flowers, buds, stalks and berries a plant) in the Flor field was 13% compared to 22% in the Zeph field (Table 7). In the Flor field the damage level seemed to be generally lower the closer to the trap the count was done, while in the Zeph field this pattern seemed more dependent on the lure. Direction did not have a significant effect ( $F_{4,145}=0.68$ ,  $P=0.61$ ) or the number of captured weevils before final count ( $F_{1,148}=2.19$ ,  $P=0.14$ ). In total, 14 weevils were captured during the five weeks of trapping. Eight of these were captured before the final counts.



**Figure 8** Cut buds per plant ( $\bar{X} \pm SE$ ) as a function of field (Florence=Flor, Zephyr=Zeph), count (first or final count), distance from the trap (in meters) and lure (plant volatile = PV, aggregation pheromone = AgPh, and both = PV+AgPh). The y-axis shows the mean number of cut buds/plant counted on four plants around each trap (c.f. Figure 4). (In the Zeph field the values for distance=0.00 only consist of one plant per lure in comparison to three plants per lure in the Flor field).

**Table 7** Overview of *Anthonomus rubi* catches in traps (N=4 per lure) baited with plant volatile (PV), aggregation pheromone (AgPh) or both (PV+AgPh). Included for both fields are also the number of total flowers/plant (mean number buds, stalks, berries and flowers per plant  $\pm$ standard error) and the number of cut buds/plant (mean number  $\pm$ standard error).

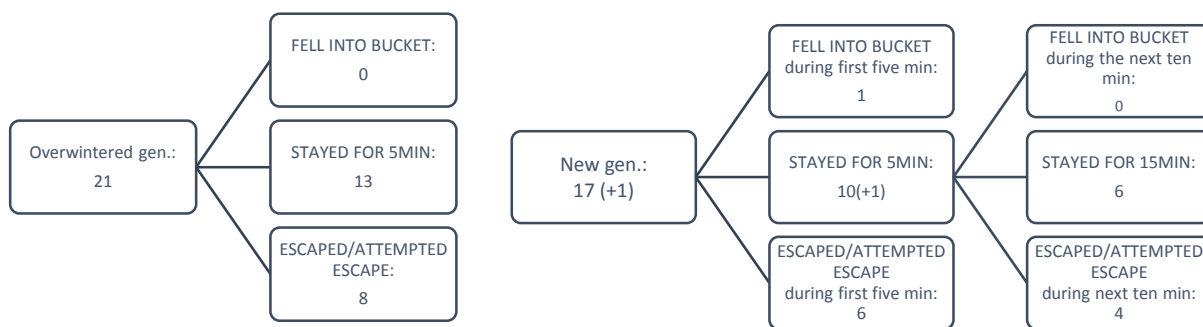
	<b>Total A. <i>rubi</i> in PV traps</b>	<b>Total A. <i>rubi</i> in AgPh traps</b>	<b>Total A. <i>rubi</i> in Pv+AgPh traps</b>	<b>Cut buds/ plant (<math>\bar{X} \pm SE</math>)</b>	<b>Total flowers/ plant (<math>\bar{X} \pm SE</math>) N=28 plants</b>
<b>The Flor field, first count (3 June)</b>	0	0	2	6 ( $\pm 0.21$ )	76 ( $\pm 1.14$ )
<b>The Flor field, final count (19 June)</b>	0	2	1	10 ( $\pm 0.26$ )	-
<b>The Zeph field, final count (3 June)</b>	1	1	1	20 ( $\pm 0.46$ )	91 ( $\pm 1.36$ )
<b>The Zeph field, after final count (19 June)</b>	0	3	3	-	-

## 3.2 Behaviour on trap

### 3.2.1 Overview of behaviour on the presumed most efficient trap for each target species

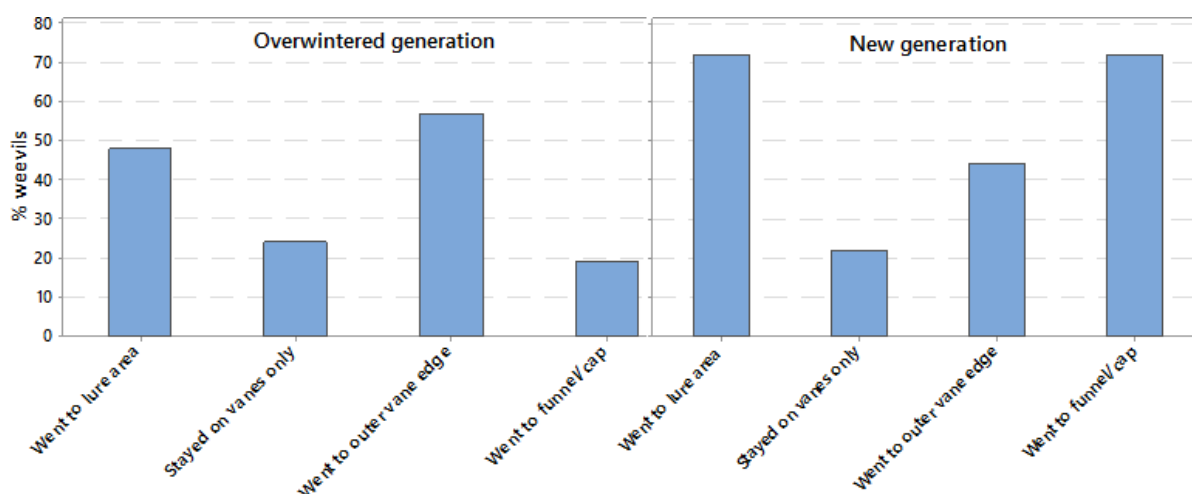
#### *A. rubi*

None of the overwintered generation *A. rubi* observed for max five minutes, fell into bucket of the funnel trap with green vanes and baited with PV+AgPh (Figure 9). Of the new generation weevils, which were observed for max 15 minutes, one weevil fell into bucket from the vanes. This was during the first five minutes (300 seconds) on trap. Of the overwintered weevils that escaped/attempted escape: 37.5% did so by flying off or opening their wings, all when standing on an outer vane edge; 37.5% went to the cap; and 25% went to the bucket. Of the new generation weevils that escaped/attempted escape, 22% flew from the funnel and 22% opened their wings on an outer vane edge. The rest ended the observation by going to the upper cap surface (33%) or to the funnel edge (22%).



**Figure 9** *Anthonomus rubi* observed on green cross vane traps baited with the combination of plant volatile and aggregation pheromone (PV+AgPh). The numbers show how many weevils were observed and their status at the end of each observation period. Overwintered generation to the left and new generation to the right. Numbers in parenthesis are new generation weevils observed in five minutes max (not included in the numbers for the next 10 minutes).

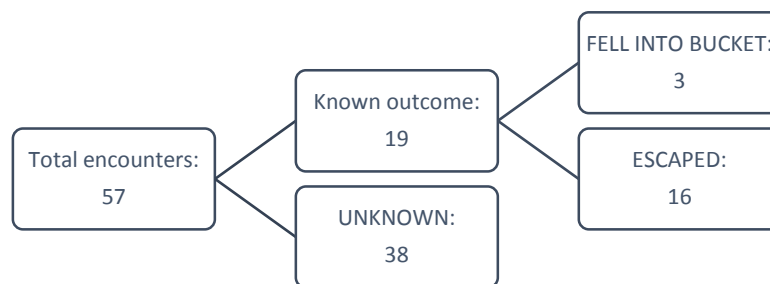
Near 50% of the overwintered weevils and more than 50% of new generation weevils were in the lure area at some point during the observation (Figure 10). New generation weevils spent most of the time on vanes: in average 65.9% (observations lasting max five minutes) and 63% (max 15 minutes) of the time on trap. (Time on vanes was not measured for the overwintered generation).



**Figure 10** Selected behavioural categories of *Anthonomus rubi* on cross-vane funnel trap and the occurrence (percentage) of weevils executing each behaviour. The observation period was five minutes after contacting the trap for both generations (overwintered weevils N=21, new generation weevils N=18). The 15 minutes observations of new generation gave a similar graph.

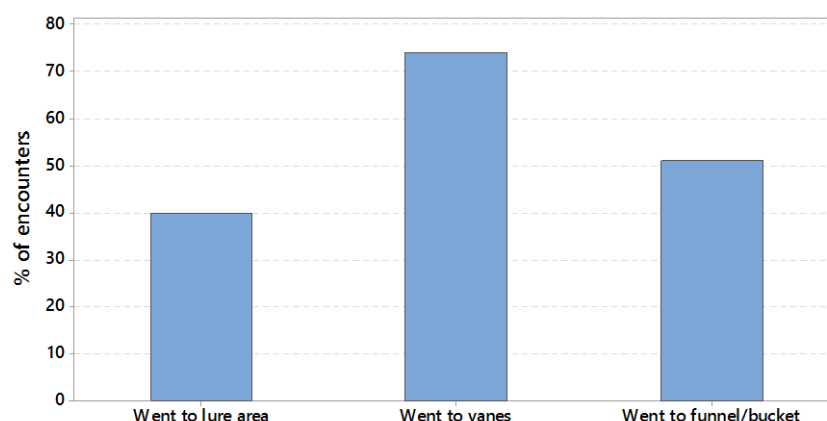
## *L. rugulipennis*

Through the 72.5 hours videotaped, 57 *L. rugulipennis* encounters were identified on the trap (Figure 11). In 66.7% of these the mirid walked out of the picture frame before it escaped or fell into bucket. Therefore the outcome was unknown. Excluding those, 15.8% encounters ended with the mirid falling into bucket. All three fell during the first five minutes.



**Figure 11** The number of *Lygus rugulipennis* that made contact with the sex pheromone baited trap, and were observed to escape or fall into bucket during an encounter. A number of mirids walked out of the picture frame, and their outcome is therefore unknown. Figure adapted from Storberget (2014).

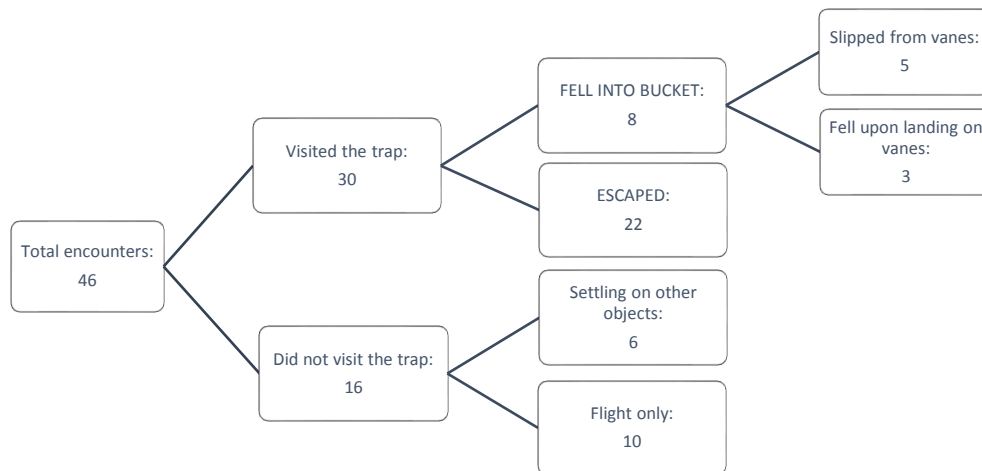
During the 57 *L. rugulipennis* encounters, 31.6% of the mirids stayed on vanes only, while 17.5% stayed on funnel/bucket only. Of the mirids that went to the vanes, 43% went to the lure area. Residence time was not measured in any category except for time on trap. The average time on trap was four minutes and 23 seconds (ranging from seven seconds to 22min and 22 sec). Figure 12 gives an overview of occurrences in the different areas of the trap.



**Figure 12** Selected behavioural categories of *Lygus rugulipennis* on funnel trap and the occurrence (percentage) of mirids executing each behaviour during an encounter.

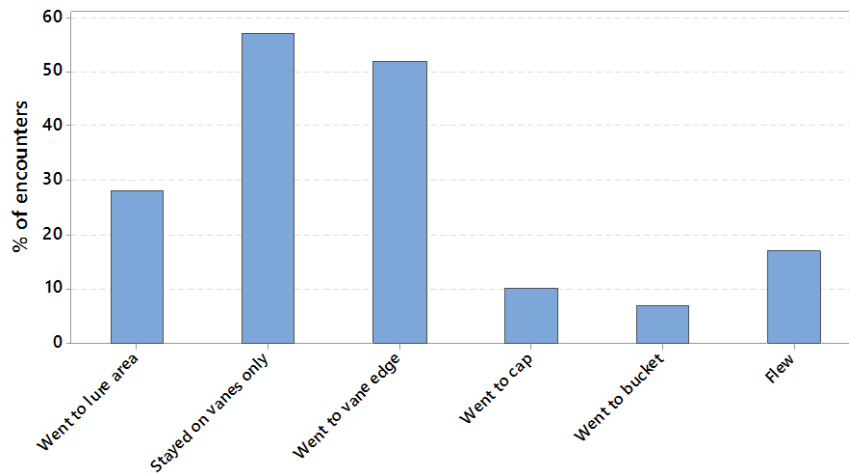
### *B. tomentosus*

During four days, about one hour in total was videotaped. All of the *B. tomentosus* that visited the trap, came flying to the trap. Of these, 27% ended the encounter by falling into bucket, while the rest escaped (Figure 13). Of the ones that escaped, 45% flew from the outer vane edge, 22% from cap, and 9% from bucket. The rest (n=4) was not filmed. Of the beetles that went to the outer vane edges, 77% escaped from this place. The beetles that fell into bucket, fell during the first five minutes on the trap.



**Figure 13** The number of *Byturus tomentosus* encounters observed within one meter of a flower volatile baited cross-vane funnel trap with white vanes, and their status at the end of each encounter. Figure adapted from Blagogie (2010).

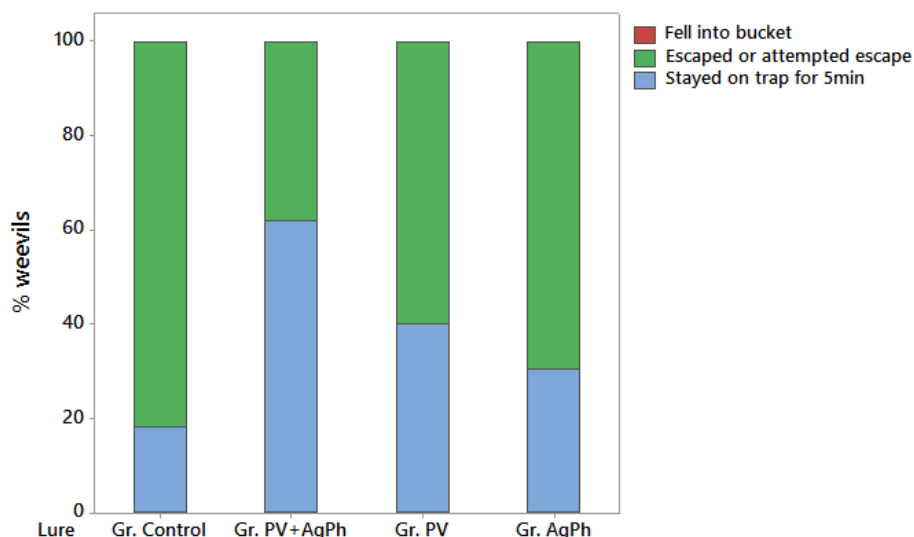
Of the *B. tomentosus* visiting the trap during the encounter, 90% landed on the vanes. The three individuals that landed on the cap flew away without seeking out any other parts of the trap. Including those, 43% of the beetles went to other parts of the trap than the vanes, while 57% stayed on the vanes only (Figure 14). In average, 80% of the time on trap during one encounter was spent on the vanes. The average time on trap was two minutes and 38 seconds (ranging from four seconds to 16 minutes, excluding one encounter in which the beetle stayed on trap for one hour and seven minutes).



**Figure 14** Selected behavioural categories of *Byturus tomentosus* on cross-vane funnel trap and the occurrence (percentage) of beetles executing each behaviour during an encounter. Figure adapted from Blagogie (2010).

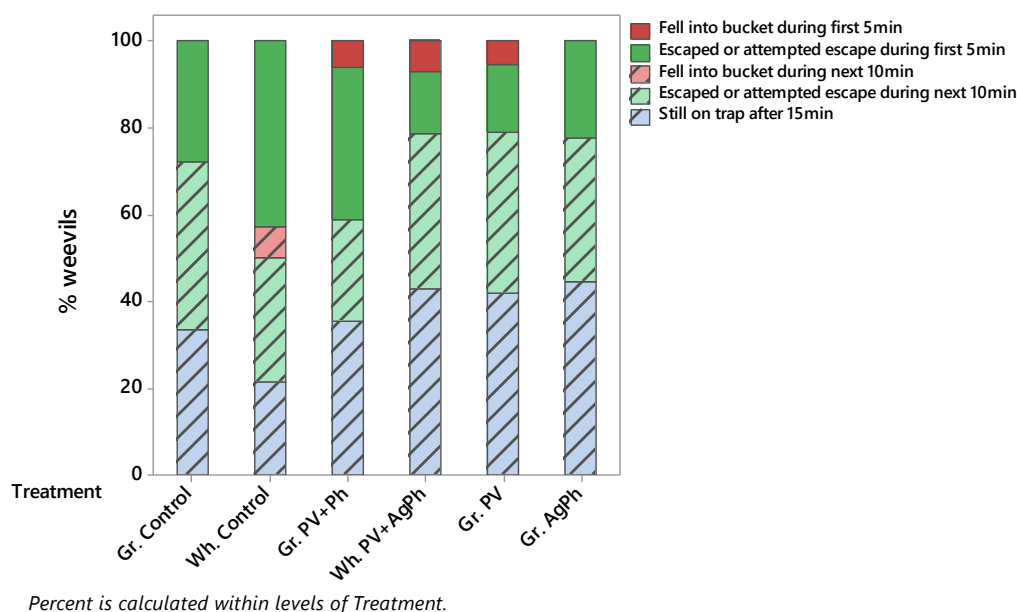
### 3.2.3 Effect of lure and vane colour on *A. rubi* behaviour

Lure had no significant effect on the status (stayed on trap for 5min or escaped/attempted escape) of overwintered weevils after five minutes observation ( $\chi^2_3=7.42$ ,  $P=0.06$ ). None of the overwintered generation weevils fell into bucket, so this response was not tested. A higher percentage stayed on the PV+AgPh baited trap for five minutes than in other treatments (Figure 15).



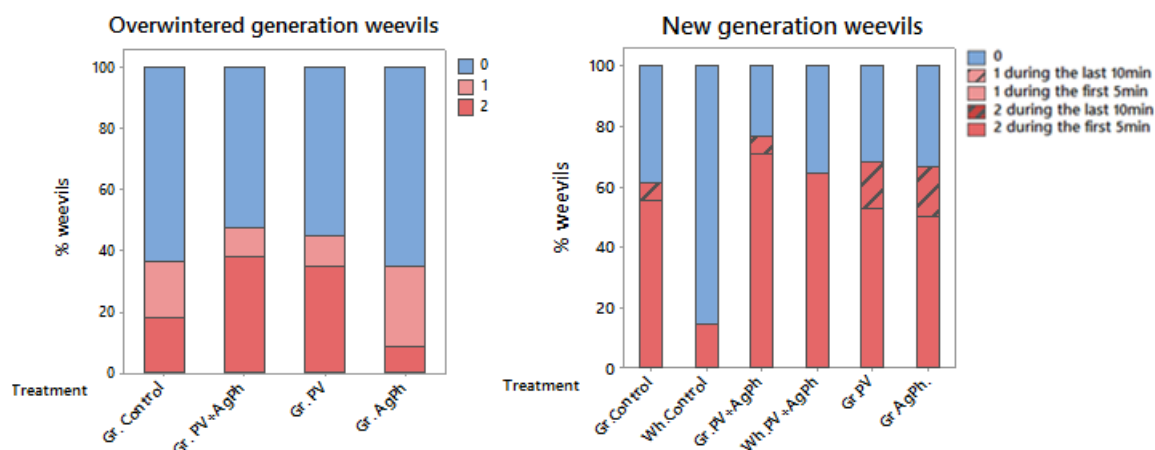
**Figure 15** Status of overwintered *Anthonomus rubi* after observations of five minutes on cross-vane funnel traps. None fell into bucket. Percentage of weevils that had either escaped/attempted escape or stayed on trap for 5min are sorted by lure used which was plant volatile (PV), aggregation pheromone (AgPh) or both (PV+AgPh). All vanes were green (Gr.).

Lure and vane colour had no effect on the status of new generation weevils (stayed on trap for 5min, stayed on trap for 15min, escaped/attempted escape) after five or 15 minutes of observation. Four weevils fell into bucket during the first five minutes, too few to use as response in a model (Figure 16). The extra time did not give any clearer differences between effect of lure and vane colour on the status at the end of the observation: one more weevil fell into the bucket, and about half of the ones that stayed on the trap for five minutes, stayed on the trap for 10 minutes more in every treatment.



**Figure 16** Status of the new generation *Anthonomus rubi* sorted by treatment (lure and vane colour) after the first five minutes of observation and after the next ten. Vanes were either green (Gr.) or white (Wh.). Lures were plant volatile (PV), aggregation pheromone (AgPh) or both (PV+AgPh). The categories concerning the minutes after the first five minutes (hatched areas) can also be read as the behavioural category “Stayed on trap for 5min”.

Lure did not have a significant effect on how many weevils showed lure oriented behaviour for the overwintered generation ( $\chi^2_3=7.07$ ,  $P=0.07$ ) (Figure 17). It did have a significant effect on time in lure area for the new generation observed for max 15 minutes ( $F_{1,96}=2.91$ ,  $P=0.04$ ) in which the control was significantly different from the rest ( $P=0.02$ ). Vane colour was not significant. Lure or vane colour had no effect on ‘stayed on vanes only’.



**Figure 17** Percentage of *Anthonomus rubi* within treatments (lure and vane colour) that went to lure area during the observations. Overwintered generation to the left and new to the right. Behaviour in lure area is sorted into three categories (c.f. Table 3): Not in lure area (0), Only approaching lure area (1), Lure oriented behaviour (2). For the new generation, category ‘1’ did not occur. The behaviours for the new generation is also categorized according to when the behaviour occurred (during the first five minutes on trap or after five minutes).

### 3.2.4 Effect of sex, trap treatment, days without food and behaviour on status at the end of the observation

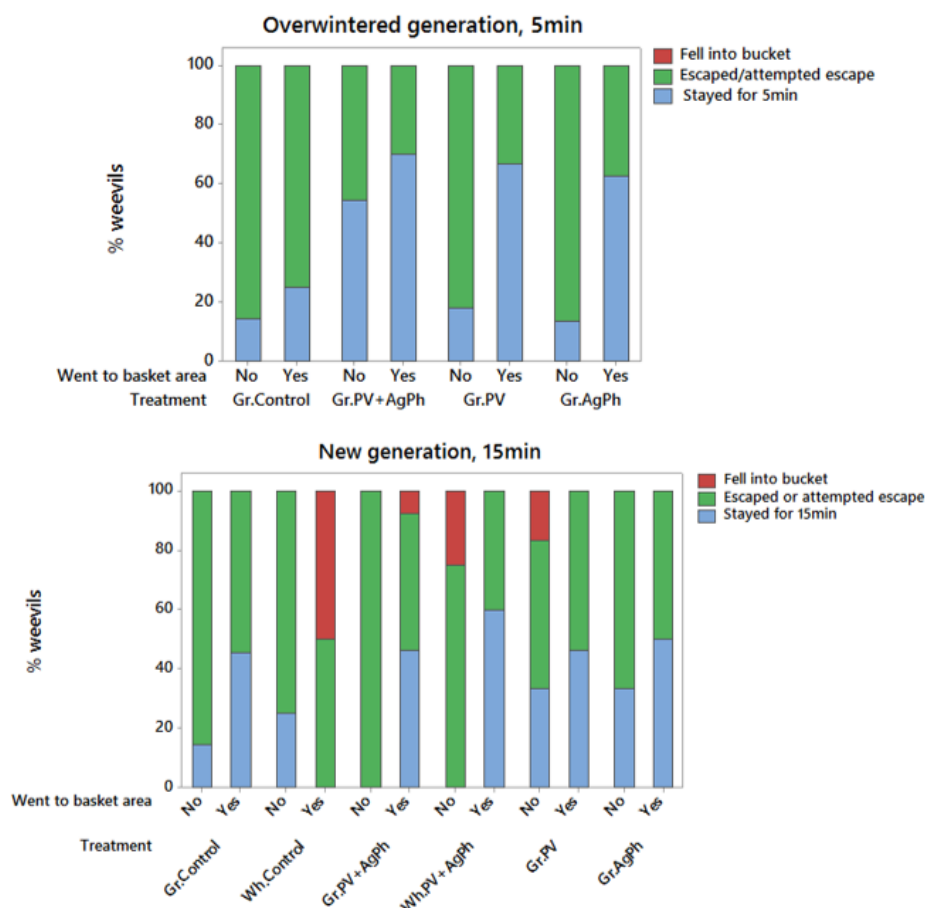
Of the *A. rubi* collected for the studies, few females (15 of 58 sexed weevils) were collected from the overwintered generation (Table 8). In the new generation, more females than males were collected (35 males and 46 females).

**Table 8** Sex ratio for the overwintered and new generation *Anthonomus rubi* used in the observation studies, sorted by treatments.

Generation	Treatment	Sex ratio		Sex unknown (weevils escaped)
		m	f	
<b>Overwintered</b>	Green control	6	2	3
	Green Pv+AgPh	9	8	4
	Green PV	12	3	5
	Green AgPh	16	2	5
<b>New</b>	Green control	3	9	8
	Green Pv+AgPh	4	8	6
	Green PV	9	9	3
	Green AgPh	8	9	3
	White control	5	6	5
	White Pv+AgPh	6	5	3



Fell into bucket were not used as response in either generation. Including lure, behavioural categories ('stayed only on vanes', 'lure oriented behaviour') and sex in the model for overwintered generation *A. rubi*, lure oriented behaviour was the only significant factor for whether the weevils had stayed for 5min or escaped/attempted escape ( $\chi^2_1=16.80$ ,  $P<0.001$ ). For the new generation, residence time in lure area had the only significant effect on stayed on trap for 15min ( $\chi^2_1=20.35$ ,  $P<0.001$ ) and stayed for 5min ( $\chi^2_1=10.32$ ,  $P<0.001$ ). None of the other factors (vane colour, stayed on vanes only, days without food, and sex) were significant. In both generations, more weevils that went to lure area stayed on trap for 5 and 15min than weevils that did not display this behaviour (Figure 18). This was the trend within every treatment for both five minutes and 15 minutes observations, except for the white control, which showed the opposite after 15min for the new generation.



**Figure 18** Status of *Anthonomus rubi* after period of observation, sorted by treatment (lure and vane colour) and the behavioural category 'went to lure area' (c.f. Table 3). Vanes were either green (Gr.) or white (Wh.). Lures were plant volatile (PV), aggregation pheromone (AgPh) or both (PV+AgPh). On top: New generation weevils observed for maximum five minutes. Bottom: New generation weevils observed for maximum 15 minutes.

For the other two other species, *L. rugulipennis* and *B. tomentosus*, none of the factors tested were significant.

## 4 DISCUSSION

### 4.1 Damage around traps targeting *A. rubi*

#### 4.1.1 Spillover effect?

The study assessing damage around traps targeting *A. rubi* did not support the hypothesis of a spillover from the traps. Distance from the trap was as expected, among the factors affecting the damage. However, the pattern was the exact opposite of a spillover effect: the damage was increasing with distance from the trap. While this was a general pattern in the Flor field, it seemed dependent on the lure in the Zeph field. In the Zeph field, damage around the PV baited trap did not show a clear pattern across the various distances, while for traps baited with the other two lures the pattern was the same as in the Flor field (with the exception of the one plant assessed at the centre by the AgPh trap). This opposite effect of a spillover is not in accordance with previous studies on *A. rubi* damage around traps baited with the AgPh (Lethmayer et al. 2004; Cross et al. 2006a). However, Lethmayer et al. (2004) and Cross et al. (2006) used variations of sticky stake traps, which could mean that the results are caused by differences in trap design. Considering the damage trend around traps only, it would seem that the cross-vane funnel traps were highly effective in capturing the species, but during the five weeks of capture, 14 weevils were captured. This number seems very low, compared to the damage. Rather, it would seem like the cross-vane funnel traps had a repelling effect on the weevils. The traps might have unfamiliar tactile cues and lack many attractants associated with a natural resource such as a strawberry plant, as Cross et al. (2006a) noted upon the low capture on sticky traps. However, it seems unlikely that visual or tactile cues would deter the weevils from 10 metres.

The semiochemicals used as lure also had a significant effect on the damage, and it seem more likely that they should affect the weevils across several metres. The highest damage was recorded around the AgPh baited traps in the Flor field and the PV+AgPh baited traps in the Zeph field. These were also the traps capturing most weevils (in the Zeph field both AgPh and AgPh+PV baited traps captured the highest number), but this was not significant. All lures have been attractive in field in previous studies (Cross et al. 2006a; Wibe et al. 2014a); in this study,

they seemed to repel the female *A. rubi*. The reason for this difference could be interactions between the lures and the natural odours in the field. The Flor field was a field with much weeds. In studies of bark beetles, non-host volatiles have been shown to deter the beetles from orientation towards attractants such as aggregation pheromones (Dickens et al. 1992; Deglow & Borden 1998; de Groot & MacDonald 1999). This repulsion of non-host volatiles was suggested to help reduce time searching for hosts and mates. Landolt et al. (1992) suggested that the combination of plant volatiles and aggregation pheromones would make the females able to discriminate against hosts of low quality. In a similar way, the combination of non-host volatiles and species specific odour semiochemicals might work as a spacing mechanism for *A. rubi*. Perhaps the ratio between non-host and host volatiles signals how likely the female is to get a bud that is not occupied. However, these speculations are not supported by previous studies of *A. rubi* distribution within a field. In which the highest numbers have been found at the edges of the crops (Aasen et al. 2004).

There is also the possibility that the position in field of the counted plants was confounding the results: plants counted furthest away from trap were also sometimes closest to the edges of the crop. (In the Zeph field, every plant at the furthest distance was close to the edge.) For this reason, a new variable was added post-hoc to the GLM model (described in section 2.4.1): plants counted closest to non-crop vegetation were coded as ‘perimeter’ while the rest were coded as ‘centre’. This new variable did not help explain the number of cut buds.

It seems most likely that the dosage or release rate of the lures used in the present study is behind the reverse spillover effect. If the release rate of this year’s lures were too high they could have repelled the weevils or disturbed the communication between them in the same way lures used for mate disruption would (Witzgall et al. 2010). Moreover, El-Sayed et al. (2006) claimed that too high dosage might lead to an optimal concentration at some distance away from the trap, which would correlate with the results in the present study.

#### 4.1.2 Differences between the two fields

The higher damage in the Zeph field compared to the Flor field support the hypothesis that older fields have higher damage because of a growing weevil population (Aasen & Trandem 2006). That these fields are of different cultivars could also have affected the damage (Łabanowska 2004). However, there was no difference between the captures in the two fields.

#### 4.1.3 Low capture

The low capture might be because of the traps disorienting or repelling effect on the weevils. It is also possible that competition with natural odours from the host plant lowered the captures as seen with *B. tomentosus* (Woodford et al. 2003; Baroffio & Mittaz 2008). If the AgPh and PV both signals mates and oviposition sites, then the traps would have had competition as long as buds were available. This is supported by the increase in capture after most of the buds had become unavailable in the Zeph field.

The lack of damaged buds around the traps does not necessarily mean there were no weevils in the vicinity of the traps as Aasen et al. (2004) collected high numbers of weevils from areas with low damage in their study. -There is still the possibility that the traps attracted the weevils without capturing them, which gives even more reason to look at what happens on the trap.

## 4.2 Observations of behaviour on trap

### 4.2.1 Capture

#### *Capture of A. rubi*

In accordance with the field study discussed above, the capture was very low (2.7% captured) in the observation studies. The five weevils all fell into the bucket when losing grip on the vanes. Since it is not unlikely that the probability of the weevils falling from exhaustion or startlement increases with time on trap, the method in the present study might be to blame for the low capture. A time limit was set in both generations, to increase the sample size and for practical reasons (the new generation weevils could stay on trap for three and a half hours, given the chance). Looking at Figure 15 the suggestion of exhaustion is not supported as all except one fell into bucket during the first five minutes. The numbers of escaped weevils however, seemed to increase gradually with time. Half of the new generation weevils that stayed for 5min had escaped/attempted escape after 15min. Thus, the time limits might not have confounded the results after all. Even so, the possibility of the weevil letting itself drop into trap when startled was supported when one of the weevils fell into bucket as it came into contact with a

smaller insect (a Diptera). Though this was after 28 seconds on the trap, the probability of this happening is very likely to increase with time.

The hypothesis that the weevils collide (i.e. slip when trying to land upon the vanes) was not tested in this study. In a pilot study containers with one weevil in each (n=32 in total) were put on the ground one at a time at different distances downwind from the trap. It quickly became clear that few of the weevils (if any) would fly to the traps: they flew past the trap and did not return, or they did not fly at all. Thus, they were placed upon the traps, and consequently, colliding was not an option. However, previous studies suggest that they do not fly to the traps, but rather climb up from the ground or from nearby vegetation (Innocenzi et al. 2001; Cross et al. 2006b); in which case, placing them at the lower part of the vanes might not have confounded the results after all.

As argued by Phillips and Wyatt (1992), details of the trap design might be discovered through direct observations. During the observations in the present study, one weevil (and two more weevils during pilot studies) fell from one of the outer vane edges and landed on the ground beside the trap. Upon closer inspection, roughly 20% of the vanes stretches outside of the funnel opening. Thus, for the falling weevils to be captured they need to fall from the inner part of the vanes (on the inner side of the holes used for the steel wires). If the weevils fall with equal probability from any part of the vanes, this means that 20% lands outside of the trap.

#### *Capture of L. rugulipennis*

Only three mirids were seen falling into the trap, while 16 escaped. It could be argued that the trapping rate should be multiplied with two because over half of the outcomes were unknown. However, it is just as likely that more were escaping from the hidden side of the trap, so the capture rate would be the same. Also, more individuals could also have landed on the other side that were not seen. Unfortunately, captured mirids was not counted afterwards, because at the establishment of the study the objective had been to learn how the mirids fell into the bucket (Fountain 2015 pers. comm.). To avoid introducing more uncertainty, the observed numbers should rather be accepted as a minimum. All observations were treated as encounters because the individuals not captured might have come back to the trap and been counted more than once.

#### *Capture of B. tomentosus*

All the *B. tomentosus* coming within one meter of the trap were also treated as encounters for the same reason as with *L. rugulipennis*. Of the *B. tomentosus* landing on the trap, 27% ended with capture (Blagogie 2010), giving the highest capture rate of this study. Half of those that fell into bucket fell from the middle where the vanes meet. The reason for this could be that the vane edges do not quite meet in the middle, but make a gap between them. This gap may function as hiding places for the beetles, as many would crawl into the gap during the observation; or difficulties upon walking from one vane to the next, as was seen in those falling from this area. One of the observed individuals fell twice from this area but saved itself by flying both times. Including more “challenges” like this in the trap design might improve the traps.

On this trap as well, roughly 20% of the vanes are outside of the funnel opening. These vanes should also be narrower as noted by Blagogie (2010).

The trap was effective in attracting the beetles coming into the area, as 65% of the filmed *B. tomentosus* visited the trap (Blagogie 2010). This is supported by Baroffio and Mittaz (2008) who discovered that the highest decrease in crop damage was around traps. The attractiveness seems to decrease with increasing distance; in a mark and recapture study by Woodford et al. (2003) sticky traps baited with flower volatiles were more effective in trapping beetles released 2.5m from the trap than beetles released from 5m. Also, it should be noted that Blagogie (2010) filmed the trap before the flowering of wild raspberry in the area. Otherwise, competition would most likely have lowered the attraction the trap (Woodford et al. 2003; Ekeland 2005; Baroffio & Mittaz 2008).

#### *Capture during the first five minutes*

For all target species the difference between individuals observed on trap and individuals captured was very high, suggesting a poor capture rate in field. However, the attraction rate in field is unknown. If the proportion of attracted individuals from a population is high, the capture rate might still be sufficient to lower the damage as seen in *B. tomentosus* (Baroffio et al. 2011). The capture rates did not improve over time: all individuals that fell into trap fell during the first five minutes (except for one single *A. rubi*), indicating that exhaustion might not be the main reason for capture. That some insects fell during the first minutes while others spent a long time walking on the vanes without falling, could suggest: the individuals got more

experienced; the individuals that fell during the first five minutes were in a poor condition; or the individuals preferred different areas of the traps, some areas which were easier to fall from (as discussed in the next section 4.2.2).

#### 4.2.2 Behaviour on trap

The behaviour that was given attention was expected to affect the capture, or behaviours that would give an indication about the movements of the insects on trap. Residence time or occurrence on vanes was chosen because vanes are where the insects are most likely to fall into bucket from. All the *A. rubi* occurred on vanes because this is where they were placed. Previous studies indicate that the area is important for the weevils. Fountain et al. (2015) found that the highest vanes gave the highest capture of *A. rubi*. The vanes seem to be important for *L. rugulipennis* and *B. tomentosus* as well, as they went here during most of the encounters. In the case of *L. rugulipennis*, half of what was seen on the video was the vanes and the other half was the funnel; the cap and the bucket was excluded from view. The high number of mirids that went to the vanes might therefore simply reflect what was seen on the video. However, 50% of the mirids occurred in the funnel/bucket area during the encounters and over 70% occurred on vanes, suggesting a preference for the vanes over the lower part of the trap. In addition, a higher percentage stayed on vanes only than on funnel/bucket only. This preference for the vanes might mean that the insects were trying to reach the odour source, but the lower occurrence in the lure area suggest that they lost the plume on the way or that the trap was saturated. As the three species feed on flowers, the upward motion might also reflect searching behaviour on a plant. However, as long as the plume leads the insects to the vanes from which they might fall into bucket, it is less important whether it leads them to the precise location of the lure.

Some areas of the trap might function as a “dead end” on trap to insects that could potentially have been captured. The few *B. tomentosus* that landed on the cap did not seek out any other areas of the trap. Since every other beetle visiting the trap went to the vanes, this either suggests that the visual cues of the vanes are crucial for capture, or that the plume did not reach the upper surface of the cap. This was also seen in the studies of *A. rubi*: the weevils that climbed on top of the cap escaped shortly after, unless they were captured by hand. In both species, the upper surface of the cap seem to be a take-off area for insects that could have been captured. Also the outer vane edges seem to be an escape route; many *A. rubi* and most of the *B. tomentosus* that escaped left the trap from an outer vane edge (neither cap nor occurrence on outer vane edges

was recorded in the behavioural sequence for *L. rugulipennis*). Almost every *B. tomentosus* that went to this area escaped.

#### 4.2.3 Factors affecting behaviour and capture

##### *Trap treatments*

When trap treatment (lure and vane colour) was tested alone, it had no significant effect on the status of weevils at the end of 5 or 15 minutes observation period. Repeatedly lure had almost a significant effect on the behaviour of the overwintered weevils. The combination of plant volatile and aggregation pheromone (PV+AgPh) gave the highest percentage of weevils staying on the trap. This was expected to be the most attractive lure because of the higher capture in previous studies (Wibe et al. 2014a). As for weevils that went to the lure area, almost as high percentage displayed this behaviour on the PV baited trap as on the PV+AgPh baited trap, suggesting PV was more important than AgPh. Perhaps because most of the individuals placed on both the PV and the AgPh baited traps were males, which were probably searching for a place to find or to signal females (Landolt 1997). Between the percentage of weevils that went to lure area on the AgPh baited trap and the control there were no difference. However, lure had no significant effect on behaviour in the lure area for the overwintered weevils. Lure did have a significant effect on the time new generation weevils spent in the lure area, but this is most likely because almost none of the weevils tested on the white control went to the lure area at all. On the green control, the percentage of weevils seeking out the lure area during 15 minutes was almost as high as those on the PV and AgPh baited traps. Vane colour was however, not significant or nearly significant ( $P < 0.1$ ), suggesting the results have to do with the individual trap or the weevils that were placed on it. The traps with white vanes had the lowest sample size (c.f. Table 4) which would have made the results from these more vulnerable to abnormal behaviour.

The lack of significance effect of vane colour was somewhat surprising. The funnel trap with white cross vanes was meant to have both the key visual and olfactory cues of a raspberry flower (Birch et al. 2008), while the green vanes are less visually conspicuous. Colour might not however be as important for *A. rubi* as for *B. tomentosus* (as the trap was designed for (Birch et al. 2008)). While *B. tomentosus* feed, mate and oviposit in open flowers (Willmer et al. 1998), *A. rubi* oviposit in flower buds which are just as visually conspicuous as the green vanes. Even so, open flowers may still be feeding and mating sites. Unfortunately, white vanes were not



included in the observations of the overwintered generation. This was reconsidered for the new generation. However, the new generation *A. rubi* emerge in July and feed mostly on foliage (Stenseth 1970), and consequently have less reasons to be affected by the visual cues of a flower. Another explanation is that visual cues do not work over short distances. When grouping stimuli according to distance, Foster and Harris (1997) categorized visual stimuli among long distance stimuli suggesting they play a role in locating the source rather than accepting it. Again, the method of this study might be blamed, as none of the weevils was allowed to try searching for the traps.

#### *Effect of behaviour on status at the end of observation time*

Neither for *A. rubi*, *B. tomentosus* and *L. rugulipennis*, could behavioural indicators for successful trapping be identified.

For *A. rubi*, the behaviour on trap was more important than sex, days without food, or treatment (lure and vane colour) for predicting whether the weevils stayed on trap for 5 or 15min. The probability of an overwintering weevil staying for 5min increased if the weevil went to the lure area, and time spent in lure area increased the probability of a new generation weevil staying for 15 min. The explanation for this may be that these weevils actually discovered the lure. However, lure was insignificant in the same model as behaviour (c.f. Figure 17). Thus, weevils seeking inwards may be less inclined to fly than the weevils seeking outwards, perhaps because of condition or other individual differences.

Sex did not affect any behaviour. In the new generation weevils this might be because they do not reproduce until next summer. In the overwintered generation the results might have been confounded by the uneven sex ratio of the tap sampled weevils. In addition, the weevils were sexed after the observations so the few females were not evenly distributed across treatments. Aasen et al. (2004) who had the same results by tap sampling, suggested males might be easier to capture by tapping because they tend to occupy the upper parts of the plants. In this case other sampling methods, such as collecting overwintering weevils, should be considered upon sampling *A. rubi* of both sexes for study.

For *B. tomentosus* and *L. rugulipennis*, none of the behavioural categories tested affected the capture rate or time spent on trap significantly. The sample size might have been too low to run statistics. Because both methods depended on individuals seeking out the trap in field, the

sample size was lower than in the *A. rubi* study. In the observations of *L. rugulipennis* the number used for analysis was lower still because of the encounters with unknown outcomes.

#### *Differences between overwintered and new generation weevils*

During the course of the present studies, several behaviour related differences between overwintered and new generation weevils emerged. Thus, it seems that behavioural indicators for capture can not be discussed without considering the differences between these two generations. The first to emerge was the numbers of weevils collected by tap sampling. While tap sampling of the overwintered generation yielded near 75 weevils in one hour, the same method and effort yielded close to none of the new generation. This may have been caused by too early sampling of the new generation, however Kovanci et al. (2005) found similar results by sweep netting which indicated a higher density of overwintered generation weevils than new generation weevils. The overwintered generation weevils might be easy to catch by these methods because of their preference for sun exposed areas (Aasen et al. 2004). While the new weevils might avoid such areas after eclosion. Willmer et al. (1996) found that newly emerged *B. tomentosus* preferred the lower areas of plants. This was because recently eclosed beetles were vulnerable to water loss and therefore avoided sun exposed areas. This might very well be the case for newly emerged *A. rubi* as well. If the new generation weevils prefer the lower areas of the plants upon emerging from the buds, they would be out of reach for both sweep netting and tap sampling, at least for a time.

The next apparent difference to emerge was the number that fell into bucket during the study. None of the overwintered fell, while five of the new generation weevils did. This is in accordance with previous studies in both Norway and Denmark, where more new generation weevils were captured than overwintered (Wibe et al. 2014a). This could have to do with winter mortality resulting in unequal population densities, but the results of Kovanci et al. (2005) and the present study suggest the numbers captured are also behaviour related. There is also the differences in biology to consider: while the overwintered generation weevils feed and reproduce; weevils of the new generation feed and overwinter. Comparing Figures 15 and 16, the overwintered generation weevils seemed to leave the trap sooner than the new generation weevils (which was also the reason why observation time was increased to 15 minutes for the new generation weevils). This might lower the probability of falling into bucket because of a disturbance. The reason for leaving sooner might be that different goals affect the searching behaviour. The overwintered weevils' goal is to maximize offspring, thus if no mate is

encountered before long they might do best in leaving for another site. The new generation seems to have no shortage in food and feeds until the end of August (Stenseth 1970), so they can afford to be less “restless”. Over half of the overwintered weevils went to the outer vane edges at some point during the relatively short observation time, which might lower the capture of overwintered weevils even more, since, as discussed previously 1) this was a preferred place to escape from; and 2) a fall from this place would not result in capture. Fewer new generation weevils went to this area. More new generation weevils went to the lure area than the overwintered, and they also seemed to stay here longer. A fall from this place would certainly result in capture.

## 5 CONCLUSIONS

### 5.1 Damage around traps targeting *A. rubi*

The spillover hypothesis was not supported as the results of the field study showed a significant increase in damage with distance from traps. This pattern was the opposite of what was expected. Efficient traps could not explain the results because very few weevils were caught. Thus, other possibilities are that the lures repelled the weevils or caused a mate disruption in the vicinity of the traps. Compared with previous studies, this would most likely be the cause of too high release rates from the lures.

The level of damage around the traps was affected by lure: the highest level was around traps baited with the aggregation pheromone alone and together with the plant volatile. This pattern could be seen at every distance that was assessed, indicating a range of attraction of at least 12.3 meters. To be certain further studies would need to be conducted. The traps would need to be positioned much further apart from each other, and the trapping continued for a longer period.

### 5.2 Observation studies

The observations of this study led to a better understanding of how the pest species behave on trap: what areas they occupy, how they are captured, how they are not captured and further, ideas on how to improve the trap.

The individuals falling into bucket were too few for identification of behavioural indicators for successful capture. Even so, the few that fell gave some indications as to how they are captured. The *A. rubi* fell into bucket by losing their footing while walking on the vanes, just as *L. rugulipennis* and most of the *B. tomentosus*. The reason for loosing grip on the vanes was mostly unknown, but one weevil seemed to let itself drop when startled. Of the captured *B. tomentosus*, 37.5% did not manage to land properly, and 37.5% fell when trying to cross from one vane to another.

Increased time on trap did not seem to increase the probability of capture for any of the three species. (However, further studies of the *A. rubi* without a time limit would need to be conducted to confirm this.) While all (except one) fell during the first five minutes, other individuals stayed on the trap for a long time without falling. This may indicate individual differences (e.g. in condition) or that the insects “learn” how to walk on the vanes without slipping. At any rate, measures should be taken to increase the probability of individuals falling and falling sooner.

Features in the trap design that might increase or decrease the capture rate were discovered. The area occupied was mostly the vanes for all species. This is an advantage for capture, should the individuals on trap fall. However, disadvantages were also discovered. The cap and the outer edges of the vanes seemed to induce flight in both *A. rubi* and *B. tomentosus* (in *L. rugulipennis* this was not considered due to lack of data concerning this). The vanes of both trap types were also too broad with 20% stretching outside of the funnel opening.

As previous studies have reported a low capture of overwintered generation weevils compared to the new generation, the behaviour of the different weevils was compared. The differences discovered might explain the previous results. The overwintered generation weevils seemed more “restless” than the new generation weevils: more overwintered weevils left before the observation time was up, and they spent more time on the outer edges on the vanes than the new generation. Both might lower the capture as less time on trap reduce the probability of falling due to a disturbance, a fall from the outer vanes will not result in capture, and the outer vanes seem to induce flight. Thus, the trap need to capture the overwintered weevils shortly after contact. In contrast, the new generation weevils spent more time in the lure area which is placed directly above the funnel opening.

Neither lure type nor vane colour seemed to make a difference for the behaviour of *A. rubi* on trap. The most surprising result was perhaps that trap treatment had no significant effect on how long the weevils stayed on trap. Thus, trap treatment most likely play an important role in attracting the weevils, but when they are on trap, the trap design will need to do the rest.

### 5.2.1 Improvements in trap design

The following are suggestions to possible improvements in trap design, based on the observations in the present study:

*Narrower vanes or wider funnel opening:* A wider funnel opening might increase the capture of other species (including mice, as have been found in the traps before) however, so narrower vanes might be the safest adjustment. Because the vanes need to be fastened to the cap and funnel there is a limit to how much of the vanes can be disposed of. This might be solved either by making holes by the funnel opening and inner part of the cap and fasten the vanes with strings (similar to an old version of the trap, but with narrower vanes); or by cutting the vanes, leaving only the place where they are fastened to the trap outside the funnel opening. Narrower vanes however, might increase the encounter rate of outer vane edges which seemed to induce flight in both *A. rubi* and *B. tomentosus*. This would have to be tested further, but a barrier or a fold at the edges might deter this behaviour. It might either lead them back, or better, make them lose their footing and fall as they try to pass it (as with the gap between vanes in the middle of *B. tomentosus* trap). Narrower vanes might also lead the insects more easily to the lure area of the trap which increased the chances of *A. rubi* staying on the trap. This might increase the capture if the vane surface was made more challenging.

*More challenging vane surface:* Slippery vanes were tested in pilot studies in both generation of *A. rubi*. Fluon was applied with a one centimetre broad brush along the edges of the vanes, including the line where the vanes meet in the middle, but excluding the part of the vanes that stretched outside of the funnel opening. Instead of applying fluon along the outer vane edge, fluon was painted in a line from top to bottom on the inner side of the holes used for steel wires. The reason for not covering the whole surface with fluon was to make it less “predictable”. Applying fluon improved the capture rate in both generations: of the 25 tested overwintered weevils, 12% fell into bucket; of the 16 tested new generation weevils, 37.5% fell into bucket. All fell from the fluon painted areas.

*Slippery inner funnel:* Some of the *A. rubi* went into the funnel without falling, and *B. tomentosus* that fell into the funnel flew back out.

*Remove barrier from the lower part of the trap:* The present trap used to catch *A. rubi* and *L. rugulipennis* has a “fold” where the funnel and bucket meet, which may act as a barrier to climbing weevils. The lower part of the trap should be more smooth like the trap that Blagogie (2010) used for *B. tomentosus* (c.f. Figure 2). If *A. rubi* enters the trap from the ground as indicated, this trap would be less challenging to climb up on.

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