



Combining plant volatiles and pheromones to catch two insect pests in the same trap: Examples from two berry crops

C.A. Baroffio^{a,*}, L. Sigsgaard^b, E.J. Ahrenfeldt^b, A.-K. Borg-Karlson^c, S.A. Bruun^{g,h}, J.V. Cross^d, M.T. Fountain^d, D. Hall^e, R. Mozuraitis^c, B. Ralle^f, N. Trandem^{g,h}, A. Wibeⁱ

^a Agroscope, Rte des Eterpys 18, 1964 Conthey, Switzerland

^b University of Copenhagen, Department of Plant and Environmental Sciences, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

^c KTH Royal Institute of Technology, Department of Chemistry, SE-10044 Stockholm, Sweden

^d NIAB EMR, New Road, Kent ME19 6BJ, UK

^e Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent, ME4 4TB, UK

^f LPPRC Latvian Plant Protection Research Centre, Struktoru lela 14 a, Riga, LV-1039, Latvia

^g NIBIO Norwegian Institute of Bioeconomy Research, NO-1431 Ås, Norway

^h NMBU Norwegian University of Life Sciences, Faculty of Environmental Sciences and Natural Resource Management, NO-1432 Ås, Norway

ⁱ NORSØK Norwegian Centre for Organic Agriculture, Gunnars vei 6, NO-6630 Tingvoll, Norway

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ABSTRACT

Most horticultural crops are attacked by more than one insect pest. As broad-spectrum chemical control options are becoming increasingly restricted, there is a need to develop novel control methods. Semiochemical attractants are available for three important horticultural pests, strawberry blossom weevil, *Anthonomus rubi* Herbst (Coleoptera: Curculionidae), European tarnished plant bug, *Lygus rugulipennis* Poppius (Hemiptera: Miridae) and raspberry beetle, *Byturus tomentosus* deGeer (Coleoptera: Byturidae). Traps targeting more than one pest species would be more practical and economical for both monitoring and mass trapping than traps for single-species. In this study we aimed to (1) improve the effectiveness of existing traps for insect pests in strawberry and raspberry crops by increasing catches of each species, and (2) test if attractants for two unrelated pest species could be combined to capture both in the same trap without decreasing the total catches. Field tests were carried out in four European countries and different combinations of semiochemicals were compared. A volatile from strawberry flowers, 1,4 dimethoxybenzene (DMB), increased the attractiveness of the aggregation pheromone to both sexes of *A. rubi*. The host-plant volatile, phenylacetaldehyde (PAA), increased the attraction of female *L. rugulipennis* to the sex pheromone, and, in strawberry, there was some evidence that adding DMB increased catches further. Traps baited with the aggregation pheromone of *A. rubi*, DMB, the sex pheromone of *L. rugulipennis* and PAA attracted both target species to the same trap with no significant difference in catches compared to those single-species traps. In raspberry, catches in traps baited with a combination of *A. rubi* aggregation pheromone, DMB and the commercially available lure for *B. tomentosus*, based on raspberry flower volatiles, were similar to those in single-species traps. In both crops the efficiency of the traps still needs improvement, but the multi-species traps are adequate for monitoring and should not lead to confusion for the user as the target species are easy to distinguish from each other.

1. Introduction

Knowledge of insect behavioural responses to combinations of semiochemicals can be used in new approaches to monitoring and trapping insect pests (Lindgren, 1983; Oelschlager et al., 1995; Suckling, 2000; Alpizar et al., 2002; El Sayed et al., 2006; Nakashima et al., 2016). Pheromone traps are already used in fruit growing areas world-wide to monitor insect pests (Tingle and Mitchell, 1975;

Suckling, 2000; Schlyter et al., 2001; Nakashima et al., 2016) but plant volatiles can potentially enhance or inhibit the response to species-specific sex or aggregation pheromones (Landolt and Phillips, 1997). The challenges are to determine the optimal blend of plant volatiles (Bruce and Pickett, 2011) and of plant volatiles and pheromones (Fountain et al., 2017; Saïd et al., 2005).

In European berry crops, plant volatiles and pheromones acting as attractants have been found for several important pest species,

* Corresponding author.

E-mail address: catherine.baroffio@agroscope.admin.ch (C.A. Baroffio).

including strawberry blossom weevil, *Anthonomus rubi* Herbst (Coleoptera, Curculionidae), (Wibe et al., 2014a), European tarnished plant bug, *Lygus rugulipennis* Poppius (Heteroptera, Miridae) (Fountain et al., 2014) and raspberry beetle, *Byturus tomentosus* DeGeer (Coleoptera, Byturidae) (Birch et al., 2009).

Anthonomus rubi is an important pest in strawberries and raspberries in northern and central Europe (Trandem et al., 2004). It overwinters as an adult and becomes active in April (Stäubli and Höhn, 1989). It feeds on leaves and flowers for two to three weeks, after which the females oviposit into flower buds and sever the petioles to protect the eggs. Each female can lay more than 150 eggs (Easterbrook et al., 2003). The larval and pupal stages develop in the cut buds. *Lygus rugulipennis* feeds on a wide range of plants and is a pest on many crops. The piercing mouthparts of nymphs and adults cause fruit distortion in up to 80% of berries (Cross et al., 2011; Fitzgerald and Jay, 2011). The bug overwinters as an adult with one to two generations in northern and central Europe. *Byturus tomentosus* overwinters as an adult, beginning activity in April and mating in May in central Europe (Antonin, 1984). In Northern Scandinavia, the beetle may need two years to complete a generation (Stenseth, 1974). Females lay an average of 100 eggs singly in flowers or young fruitlets with an average of 1–3 eggs per day (Antonin, 1984). Damage is caused by larval development in fruits, and the tolerance of retailers is often zero, i.e. fruit consignments are rejected if one larva is found (Schmid et al., 2006).

These three pests are commonly controlled using broad-spectrum chemical insecticides that may have negative impacts on the environment, non-target insects and human health, and their use is increasingly restricted (Hillocks, 2012). Furthermore, *A. rubi* is developing resistance to insecticides (Aasen and Trandem, 2006), limiting the effect of chemical control. There is also a high demand from consumers for fruits without pesticide residues (Tamm et al., 2004; Cross and Berrie, 2006). Thus growers, especially organic growers who have few effective measures against such pest insects, require alternative methods for pest management. The use of traps baited with attractants can be part of a control strategy aimed at managing pest insects without using synthetic insecticides. This has previously been shown for the boll weevil, *Anthonomus grandis grandis* (Boheman) in U.S. cotton, where pheromone trapping has been important both for monitoring and control (Ridgway et al., 1990).

Innocenzi et al. (2001, 2002) identified four components of the male-produced aggregation pheromone of *A. rubi*, and the lure was further optimized by Cross et al. (2004; 2006a, 2006b). More recently, Wibe et al. (2014a) reported that addition of 1,4-dimethoxybenzene, a component of the volatiles from the flowers of wild varieties of strawberry, increased the attractiveness of the pheromone blend to *A. rubi*.

Components of the female-produced sex pheromone of *L. rugulipennis* were identified as hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal by Innocenzi et al. (2005). A precise blend of these chemicals released from specially-developed dispensers was shown to attract males to traps in the field (Fountain et al., 2014). An effective trap should also attract females, as do aggregation pheromones and plant volatiles (El-Sayed et al., 2006). Frati and Salerno (2008) found that both males and females of *L. rugulipennis* were strongly attracted to host plants with conspecific females in laboratory bioassays, suggesting that host-plant volatiles increased the attractiveness of the pheromone. Some of these volatile compounds were identified and phenylacetaldehyde (PAA) was shown to increase the attractiveness of the sex pheromone (Frati et al., 2009; Koczor et al., 2012).

Byturus tomentosus uses both visual and olfactory cues to locate raspberry flowers (Woodford et al., 2003), and two volatile components (RV) of the raspberry flower odour plume are particularly attractive (Woodford et al., 2003). A trap based on the most attractive blend was developed in Scotland and tested in Scotland, Norway and Switzerland (Birch et al., 2009; Trandem et al., 2009; Baroffio and Mittaz, 2011; Baroffio et al., 2012).

Currently, the semiochemical traps are only used for monitoring these three pests, but they have potential for controlling the pests by mass trapping if they can be further optimized. For example, by adding plant volatiles female insects searching for egg-laying sites may be attracted and trapped, thereby reducing crop damage (Wibe et al., 2014b; Fountain et al., 2015; Trandem et al., 2015). Also, insect traps would be more cost effective if each trap attracted more than one pest species, reducing the material and labour costs of maintaining separate traps for each pest (Wibe et al., 2012). Such combination traps are feasible if the semiochemicals used to attract one target species do not significantly reduce the attractiveness of those for another species. Insect pheromones are species-specific and interference between pheromones is unlikely as Smit et al. (1997) demonstrated for sweet potato weevils, *Cylas puncticollis* (Boheman) and *C. brunneus* (Fabricius) (Coleoptera: Curculionidae). However, adding plant volatiles to a pheromone may disrupt the behavioural response of the insects to the pheromone (Wang et al., 2016).

In the present field studies we aimed to (1) improve the effectiveness of existing monitoring insect traps in strawberry and raspberry by increasing catches of both sexes of the three target species, and (2) to demonstrate that two unrelated pest species could be attracted by semiochemicals to the same trap without decreasing the total catches. The possible influence of background volatiles on trapping success (Cai et al., 2017; Knudsen et al., 2017), made it preferable to carry out experiments in the field rather than the laboratory.

2. Materials and methods

The experiments were carried out during 2013 and 2014 in UK, Denmark, Norway and Switzerland (see Table 1). For the strawberry pests, *A. rubi* and *L. rugulipennis*, semiochemical blends targeting single species were tested in 2013, and in 2014 the concept of a single trap targeting two species was tested. For the raspberry pests, *A. rubi* and *B. tomentosus*, semiochemical blends had already been tested separately in previous studies (Baroffio et al., 2012; Wibe et al., 2014b) and thus the concept of using a single trap for the two species was studied in both years.

3. Traps

In all experiments green funnel traps (Unitrap) with cross-vanes were used (Agrisense, Treforest, Pontypridd, UK, and Sentomol, Glen House, Monmouth, UK). These traps consisted of a bucket (16 cm diameter, 12.5 cm high), with a funnelled entrance (3 cm diameter), beneath pointed cross vanes (10 cm high) with a circular lid (16.5 cm diameter) over the top. The bucket was filled with 500–700 ml water containing 0.1% Triton X-100 to reduce the surface tension and drown insects falling through the funnel. In tests in strawberry, cross-vanes were green and in tests in raspberry they were white, acting as a visual attractant for *B. tomentosus*.

Parallel work on trap design in strawberry showed the Unitrap with green cross vanes to be more effective than other designs of trap (Fountain et al., 2017). The white colour attracted bees, thus a bee excluder grid above the funnel entrance that allows bees to fly off the trap was used (Trandem et al., 2009; Baroffio and Mittaz, 2010). Traps were placed on the ground among plants and secured by a bent welding rod (Wibe et al., 2014b) or hung 50 cm above ground on a wooden pole in some raspberry experiments. Traps were emptied and the water renewed every 14 days, at the same time removing foliage entangled in the vanes. Traps were renewed each year to avoid contamination from lures used in the previous year.

3.1. Semiochemical lures

Dispensers with sachets or polyethylene vials containing semiochemicals targeting each species individually were fastened under the

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