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Potential for Attractive Semiochemical Lures in *Rhagoletis cerasi* (L.) Management: A Field Study

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ABSTRACT

In the view of sustainable management of *Rhagoletis cerasi* (L.) (Diptera, Tephritidae), a key pest of cherry orchards in Europe, we tested the efficacy of five blends containing male produced volatiles that were used as lures on yellow sticky traps, during a two year field study. Results show a superior efficacy of one of the blends (RC1 = 2-hexanone: 3-heptanone: nonanal), which captured three times more individuals than control in both years. Good results were also obtained by RC2 (beta-phellandrene: geranyl acetate: (+)-limonene) with an average number of catches which was half that of RC1 in both years. Our findings showed that one of the tested blends, which possibly represents the male sexual pheromone, has a promising potential for practical applications of synthetic lures in monitoring, mass-trapping or attract and kill strategies.

Key words: Attractant volatiles, European cherry fruit fly, integrated pest management, pheromones, *Tephritidae.*

INTRODUCTION

Rhagoletis cerasi Loew (Diptera, Tephritidae) is a key pest of Romanian cherry orchards (Chireceanu, 2008) and also of other European countries (Daniel and Grunder, 2012). Depending on the cultivar, infestation level of fruits can vary from 60% (Bandzo *et al.,* 2012) up to harvest jeopardizing (Fimiani and Cavalloro, 1983), especially in biological agrocenoses (Letcheva *et al.*, 2001).

The reliance on chemicals is often the most used management strategy. Given the side effects such as residual ecotoxicity to beneficial organisms and humans, cherry production is challenged by the withdrawal of insecticides in many European countries (Daniel, 2009). Therefore, *Rhagoletis cerasi* management is now focused on sustainable and effective strategies.

Among the different alternatives being developed, the usage of semiochemicals is of particular interest, especially for their suitability in integrated pest management programs (Baker, 2009). Although their identification is challenging (Wehrenfennig *et al*., 2013), volatile compounds have been studied in more than 30 tephritid species of economic interest (Wicker-Thomas, 2007). Potential applicability of these semiochemicals in integrated management strategies has been reviewed (Benelli *et al*., 2014; Jeyasankar, 2009; Sivinski and Calkins, 1986). Among the methods involving semiochemicals in fruit flies control, a successful case was the combined use of sexual pheromone and food attractants in monitoring and mass-trapping of *Bactrocera oleae* (Bueno and Jones, 2002). Male lures (Tan *et al*., 2014) are also effectively used in mass trapping of Mediterranean fruit fly (Trimedlure) or in attract and kill strategies for Melon fly ("cue-lure") and Oriental fruit fly (Methyl eugenol) (Witzgall *et al*., 2010).

The use of attractive semiochemicals for fruit flies has also been explored in terms of biosecurity (Suckling, 2015), biological control mediated through bacteria semiochemicals (Leroy *et al*., 2011), or to switch on plant defense by attracting the natural antagonists (Pickett *et al*., 2012). In case of eradication programs (Suckling *et al., 2014)*, the availability of specific lures will substantially increase the success (Tobin *et al*., 2014).

The use of host marking pheromones allowed to achieve significant reductions in the pest incidence for *Ceratitis capitata* (Arredondo and Díaz-Fleischer, 2006) and *Anestrepha obliqua* (Aluja *et al*., 2009). Applicability of these semiochemicals in mass rearing process of the specific parasitoids has been suggested by Silva *et al*. (2012).

Regarding *Rhagoletis* genus, previous studies have shown that *R. pomonella* is efficiently attracted by parapheromones such as butyl hexanoate (Bostanian and Racette, 2001) and by food-attractants like ammonium carbonate (Yee *et al*., 2014). This type of volatiles can also be used in combined strategies for reduced-risk insecticide applications (Pelz-Stelinski *et al*., 2006) or in addition to monitoring traps (Pelz-Stelinski *et al*., 2005), where the use of ammonium acetate increased captures of *R. cingulata*.

Currently monitoring for the European cherry fruit fly *R. cerasi* is performed by means of yellow sticky traps ("Rebell" type), and Katsoyannos *et al.* (2000) showed that adding the food attractant ammonium acetate increased trap efficiency. However, the use of other types of attractants has never been considered and relatively few studies are available, possibly due to difficulties in identification and formulation of the components, but also for the difficulties in rearing this species (Daniel and Grunder, 2012). Guerin *et al.* (1983) studied the response of several fruit flies to volatile substances that are known as "general green leaf volatile complex" substances, however, none of them was attractive to *R. cerasi*. Later, seventy-eight generally occurring plant volatiles and male cherry fruit volatiles were investigated by means of olfactory selectivity (Raptopoulus *et al*., 1994-1998). In terms of kairomones, methyl salicylate was shown to affect the fruit fly oviposition (Köppler *et al.*, 2010). The oviposition host marking pheromone was successfully identified and obtained good results in the field (Aluja and Boller, 1992). Although the existence of an attractant sexual pheromone was mentioned (Katsoyannos, 1982), its exact composition has not been identified yet (Daniel and Grunder, 2012). Raptopoulos *et al*. (1995) later identified a high number of the volatiles emitted by *R. cerasi* males and after performing behavioral bioassays he obtained EAG responses on female antenna.

The aim of this study was to determine the effectiveness of different semiochemical blends in field conditions for the European cherry fruit fly, using as a starting point the most attractive substances based on EAG responses, as identified by Raptopoulos *et al*. (1995), considered as attractant pheromones also by Wicker-Thomas (2007) and in the specific pheromone database (El-Sayed, 2014).

MATERIALS AND METHODS

The experiment was carried out during two years in an organic cherry orchard (9 ha size), located in Cluj-Napoca city, Romania, with plants from mixed cherry cultivars (Ramon Oliva, Boambe de Cotnari, Germersdorf, Van, Stella and Rubin) randomly distributed all over the field. No chemical treatments were performed.

The chosen volatile compounds, based on Raptopoulos *et al*. (1995) were: 2-hexanone, 3-heptanone, nonanal, beta-phellandrene, geranyl acetate and (+)-limonene (Fig. 1).

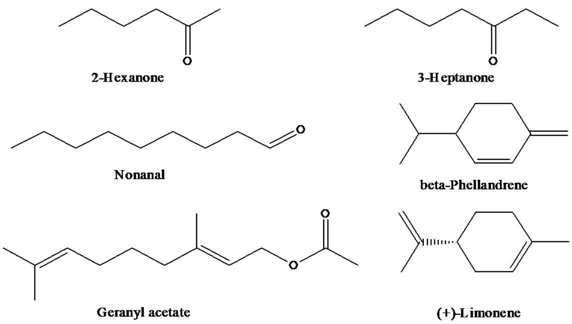

Fig. 1. The structure of the pheromonal components of *Rhagoletis cerasi* males, tested in the experiment.

The five experimental lures consisted of blends of the six selected compounds considering the two groups carbonyl (ketones and aldehydes) and terpenes, and combinations of substances belonging to the two groups, using different proportions. Based on this, the following blends were tested:

Lure RC1 = 2-hexanone: 3-heptanone: nonanal = 1:1:2 = 1mg/lure;

Lure RC2 = beta-phellandrene: geranyl acetate: (+)-limonene = 1:1:1 = 1.5 mg/lure

Lure RC3 = 2-hexanone: 3-heptanone: nonanal: beta-phellandrene: geranyl acetate = 1:1:1:1:1 = 1mg/lure;

Lure RC4 = 2-hexanone: 3-heptanone: nonanal: beta-phellandrene: geranyl acetate: (+)-limonene = 1:1:1:1:1:1 = 1.2 mg/lure;

Lure RC5= 2-hexanone: 3-heptanone: nonanal: beta-phellandrene: geranyl acetate = 1:1:1:1:1 = 2.5 mg/lure.

Each lure was dissolved in a solution of (50 ml) n-hexane, incorporated in a rubber septa (15 mm diameter) (Romfarmachim, Romania) and then attached to the upper part of a yellow sticky trap. The red sleeve stopper was packed after the evaporation of n-hexan.

The control consisted of a rectangular yellow sticky trap with double sided adhesive (210mm x 297mm) (Isabella yellow, Poliam Cluj-Napoca, Romania).

The traps were placed at an approximate height of 1.7-1. 9 m, on the southern part of canopy, at a minimum distance of 20 m one from each other. Eight replicates for all five variants and eight replicates for control were set up. Traps reading was performed twice a week (flies were not sexed) from the beginning until the end of *R. cerasi* flight, respectively between end of May to early July in 2013 and third week of May to beginning of July in 2014. During each experimental period, no changes of lures were performed, per manufacturer's instructions.

The substances 2-hexanone 98%, 3-heptanone 98% and nonanal 97% were purchased from Alfa Aesar (Romania); Beta-phellandrene and (+)- limonene was purchased from SC Terpena SRL (Romania). Geranyl acetate 98% was synthesized in the laboratory of "Raluca Ripan" Institute for Research in Chemistry by acetylation of geraniol 97% (Sigma Aldrich) with acetic anhydride in pyridine at reflux, purified by silica gel (n-Hexane:Diethylether = 10:1) and analyzed by GC-MS analysis.

Due to heteroscedasticity, the differences in the numbers of insects captured using the different lure blends were compared, separately for each year, using non parametric Kruskal-Wallis analysis of variance, followed by post-hoc comparisons of mean ranks of all pairs of groups. Statistical tests were performed with STATISTICA 7.1 (StatSoft, 2005).

RESULTS

The results show that in year 2013 highly significant differences were detected in the number of cherry fruit flies catches ($H = 31.49$, $N = 48$, $P < 0.0001$). Multiple comparisons showed that lure RC1 captured a significantly higher ($P < 0.001$) number of flies compared to Control ($z = 3.51$, $p = 0.006749$), RC4 ($z = 4.96$, $p = 0.000011$) and RC5 ($z = 4$, $p = 0.000987$), whereas no differences were detected between RC1, RC2 and RC3 (Fig. 2). In addition, no significant differences were detected between RC4, RC5 and Control.

During the year 2014, highly significant differences ($H = 17.05$, $N = 48$, $P = 0.004$) were observed, with multiple comparisons showing that RC1 captured a higher number of flies compared to Control ($z = 3.018$, $p = 0.038185$), RC3 ($z = 3.11$, $p = 0.028336$), RC4 ($z = 3.08$, $p = 0.031013$) and RC5 ($z = 3.47$, $p = 0.007714$) (Fig. 3). No differences were detected between RC1 and RC2. Control was not significantly different from RC3, RC4 and RC5.

DISCUSSION AND CONCLUSIONS

Our study was aimed at comparing different semiochemical lures for attracting the European cherry fruit fly by means of a field trial performed over two years. We used male produced volatiles that were shown to be the most attractive components in laboratory bioassays (Raptopoulos *et al*., 1995). One of the lures, RC1, showed the best performance in both years with attraction to it three times greater than to the control. RC2 lure showed good potential also, with an average number of catches which was half that of RC1 in both years. In studies concerning the male volatiles of *C. capitata,* in laboratory experiments, blends with the five major components were attractive, though not as efficient as live males (Jang *et al*., 1994). In field tests the use of mixtures with the three male emitted major components proved to be attractive in Guatemala (Heath *et al*., 1991), whereas similar trials performed in Spain gave disappointing results (Howse and Knapp, 1996). On the other hand, the use of single components from male borne emissions showed unsatisfactory results for this fruit fly both in laboratory (Jang *et al*., 1994) and in field studies (Delrio and Ortu, 1988).

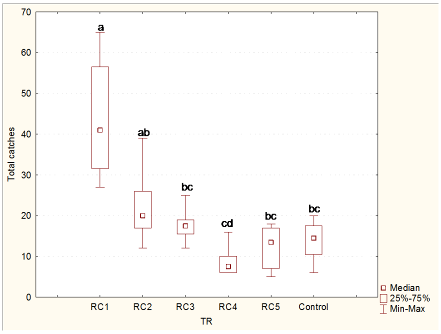

Fig. 2. Box and whisker plot representing the captures of *Rhagoletis cerasi* during 2013 in the traps baited with the different semiochemicals blends or in unbaited traps (control). Boxes indicated with the same letters are not significantly different after the Kruskal-Wallis analysis of variance, followed by post-hoc comparisons of mean ranks of all pairs of groups.

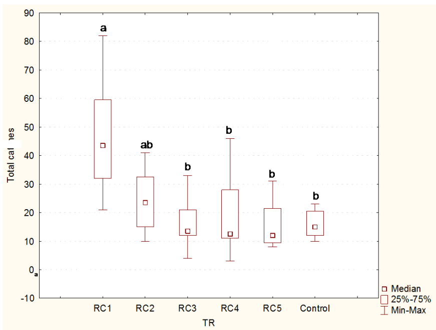

Fig. 3. Box and whisker plot representing the captures of *Rhagoletis cerasi* during 2014 in the traps baited with the different semiochemicals blends or in unbaited traps (control). Boxes indicated with the same letters are not significantly different after the Kruskal-Wallis analysis of variance, followed by post-hoc comparisons of mean ranks of all pairs of groups.

RC1 consisted of a blend of 2-hexanone, 3-heptanone and nonanal and RC2 in beta-phellandrene: geranyl acetate: (+)-limonene. The RC1 and RC2 lures contain different groups of chemical compounds, with the first blend containing only carbonyl compounds and the second only terpenoid compounds. The attractiveness of these two groups used separately showed greater efficacy than the control whereas, when these groups were mixed in different proportions for the other three blends, the attractiveness was significantly diminished. Therefore, it appears that terpenoid and carbonyl compounds do not have a sinergistic action in attracting *R. cerasi,* suggesting that together their positive effects are cancelled out.

The components of RC1 blend are utilized in chemical communication systems of a relatively small number of insects. Focusing on Diptera species, heptan-3-one and hexan-2-one are reported as pheromones only for *R. cerasi* (El-Sayed, 2014). Nonanal is reported as a kairomone for mosquito species, eg. *Culex quinquefasciatus* (Irish *et al*., 2014) and *Culex tarsalis* (El-Sayed, 2014), and for the fly *Atherigona soccata* (El-Sayed, 2014) and represents a pheromonal component for another fruit fly pest, *Bactrocera oleae* (El-Sayed, 2014). Field test with nonanal used as a single component resulted in failure of attraction for *R. cerasi* (Guerin *et al*., 1983). Regarding RC2 lure, its components are involved in chemical communication of a higher number of insects, among which Tephritidae family is represented by three more species beyond *R. cerasi, Anastrepha fraterculus, A. ludens* and *C. capitata* (El-Sayed, 2014). Therefore, the blend of lure RC1 presents a potential for fruit fly selectivity.

In our study, the blends RC3, RC4 and RC5 that contained both heptan-3-one and geranyl acetate were not attractive, as compared with no odor in the controls. These substances, detected among the major *R. cerasi* male volatiles (Raptopoulos *et al*., 1995) have been identified also in its host plant (El-Sayed, 2014; Mattheis *et al*., 1992). Therefore, a possible explanation for the lack of attraction of these lures is the competition with the natural host odors. Similar hypothesis were made also by Howse and Knapp (1996) regarding failure of attraction of *C. capitata* male volatiles competing with citrus host in field trials. An alternative hypothesis is that these substances need to be part of a blend not yet identified, in particular ratios not yet tested.

Our study tested only part of the major compounds identified from the male-produced odor range (Raptopoulos *et al*., 1995) and it is possible that the use of different blends with the identified components could achieve better results. Nevertheless, we demonstrated for the first time that a male derived semiochemical blend obtained very good results in attracting *R. cerasi* in the field. Due to the fact that in this fruit fly no male to male attraction or male to female attraction was observed (Katsoyannos, 1982), it is likely that a great number of the individuals captured with RC1 lure were females and therefore this blend could represent the male sexual pheromone.

Having a minimal impact on the environment and on beneficial organisms (Wehrenfennig *et al*., 2013) the application of semiochemicals has been an integral part of sustainable pest management programs for more than 40 years, being used in several types of controlling actions (Baker, 2009). Regarding European cherry fruit fly management, our findings showed that one of the tested blends could represent a promising candidate for practical applications of synthetic pheromone lures. Further research is needed to investigate its role as a complementary tool to improve monitoring for *R. cerasi*.

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