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Behavioral Effect of Volatile Organic Compounds on Codling Moth Larvae *Cydia Pomonella*

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Abstract

The behavioral effect of 4 mixtures volatile organic compounds tested separately is evaluated on *Cydia pomonella* codling moth larvae. The average attractiveness of the neonate larvae of codling moth varies according to the mixtures tested; it decreases proportionally with the increase of the doses used and the duration of experimentation. This study shows that neonate larvae have a preference between the tested mixtures, it shows an attractiveness for both mixture B and D, but seem less attractive by mixtures A and C. Variance analysis with two classification criteria revealed no significant differences for the organic compounds factor and significant differences for the dose factor. The test of Newman and Keuls at the threshold of 5% highlights two homogeneous groups: A and B

Keywords:
Cydia pomonella, volatile organic compounds, behavior, attractiveness.

1. INTRODUCTION

Insects tend to choose their host plants with predilection; they are able to detect them even if they are hidden in a floristic processionand this due to volatile organic compounds released by the plant. They play a key role in the identification of the host location and recognition. These are olfactory signals that make plant-insect interactions specific (Witzgall and *al*., 2005).Some of these volatile substances act as olfactory stimuliwhich play an important role in research of the host by phytophagous larval insects (Mondy and *al*., 1998; Singh and Mullick, 2002).

Larvae must find feeding sites after hatching; they are generally attracted by the volatile matter of the host plants (Jones and Coaker, 1978; VisserandAvé, 1978; Visser, 1986).

The objective of this study is to determine the olfactory response of neonate larvae of *C. pomonella* codling moth to volatile organic compounds,which could lead to the development of crop protection strategies against insect pests by modifying the behavior of the herbivore in favor of the host plant.

2. MATERIALS AND METHODS

2.1. Insects

To guide an appropriate control method against apple codling moth, a study of its behavior is carried out in the laboratory. Insects used for experiments come from a population of chrysalis and eggs of *C. Pomonella* from breeding on an artificial environment from society NPP-Arysta Life Science. The larval attraction test is carried out with batches of 10 neonate larvae.

Adult codling moths are sexed as pupae and that by differentiating the genital opening in the last abdominal segments. Adults are placed in nest boxes to obtain eggs and pregnant females.

2.2. Volatile organic compounds

During this study tests are carried out with different synthetic compounds that are diluted to obtain the following concentrations 1µg/µl, 5µg/µl, and 10µg/µl. The formulations are as follows:

- β -Caryophyllene, Cis-3-Hexen-1-ol, Farnesene (A)
- β -Caryophyllene, Cis-3-Hexenyl acetate, β-Farnesene (B)
- β -Caryophyllene, Cis-3-Hexen-1-ol, β-Farnesene (C)
- β -Caryophyllene, Cis-3-Hexenyl acetate, Farnesene (D)

We assigned them letters to distinguish them and facilitate the transcription and interpretation of results; we add numbers to define the different concentrations: 1 corresponds to 1µg/µl, 5 correspond to 5µg/µl, and 10correspond to 10µg/µl.

2.2. Larval attraction tests

In our case the odor source is deposited on small pieces of filter papers at the other end of the petri dish. 10 larvae are used for each test, for each compound and four repetitions are carried out at the rate of one larva per petri dish.

Behaviors are filmed by a camera type Hobby Velleman Digital USB microscope N: 130907, cust: VCC37506 (1280 x 1024) Pixelsand timed by an electronic timer type Supelco cat N: 400 31x.The effect of four mixtures separately (β -Caryophyllene, Cis-3-Hexenyl acetate, β-Farnesene; β-Caryophyllene, Cis-3-Hexenyl acetate, Farnesene ; β-Caryophyllene, Cis-3-Hexen-1-ol, β-Farnesene ; β-Caryophyllene, Cis-3-Hexen-1-ol, Farnesene) à at different doses of: 1µg/µl, 5µg/µl et 10µg/µlare tested. The attraction is considered as a displacement directed towards the source of smell.

2.3. Statistical analysis

The results obtained on the attractiveness of neonate larvae of *C. pomonella* by volatile organic compounds are subjected to a multi-criteria variance analysis at the threshold P=5%, using the software STAT BOX, version 6.4. When the probability (P) is:

P> 0.05: the variables show no significant difference.
P< 0.05: the variables show a significant difference.
P< 0.01: the variables show a highly significant difference.
P< 0.001: the variables show a very highly significant difference.

When this analysis reveals significant differences, it is supplemented by the Newman and Keuls test at the 5% threshold, which makes it possible to determine the homogeneous groups.

3. RESULTS

The results obtained for the action of various volatile organic compounds of different mixtures at different doses towards attractiveness of larvae of codling *C.pomonella* apple are elucidated. The results obtained for the action of compound A (β -Caryophyllene, Cis-3-Hexen-1-ol, Farnesene) tested against neonate larvae of *C. pomonella* at different doses (1µg/µl, 5µg/µl, and 10µg/µl) are illustrated in Figure 1. The results obtained for the action of compound B (β -Caryophyllene, Cis-3-Hexenyl acetate, β-Farnesene) tested against neonate larvae of *C. pomonella* at different doses (1µg/µl, 5µg/µl, and 10µg/µl) are illustrated in Figure 2. The results obtained for the action of compound C (β-Caryophyllene, Cis-3-Hexen-1-ol, β-Farnesene) tested against neonate larvae of *C. pomonella* at different doses (1µg/µl, 5µg/µl, and 10µg/µl) are illustrated in Figure 3. The results obtained for the action of compound D (β -Caryophyllene, Cis-3-Hexenyl acetate, Farnesene) tested against neonate larvae of *C. pomonella* at different doses (1µg/µl, 5µg/µl, and 10µg/µl) are illustrated in Figure 4.

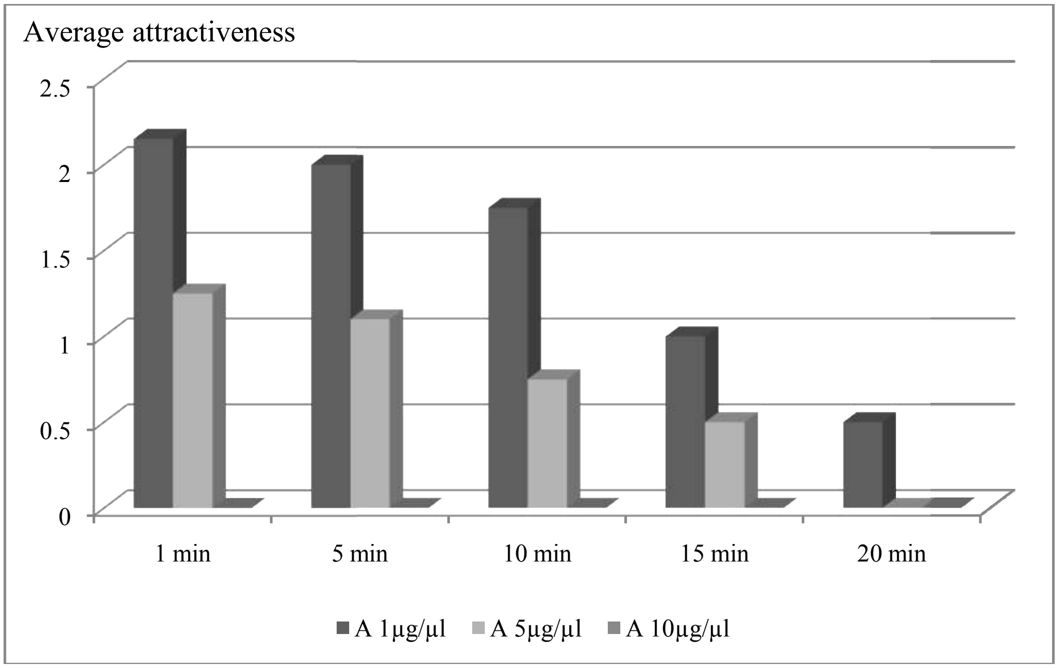

Figure 1: **Attractiveness of neonate larvae by the action of compound A at different doses (1µg/µl, 5µg/µl, and 10µg/µl).**

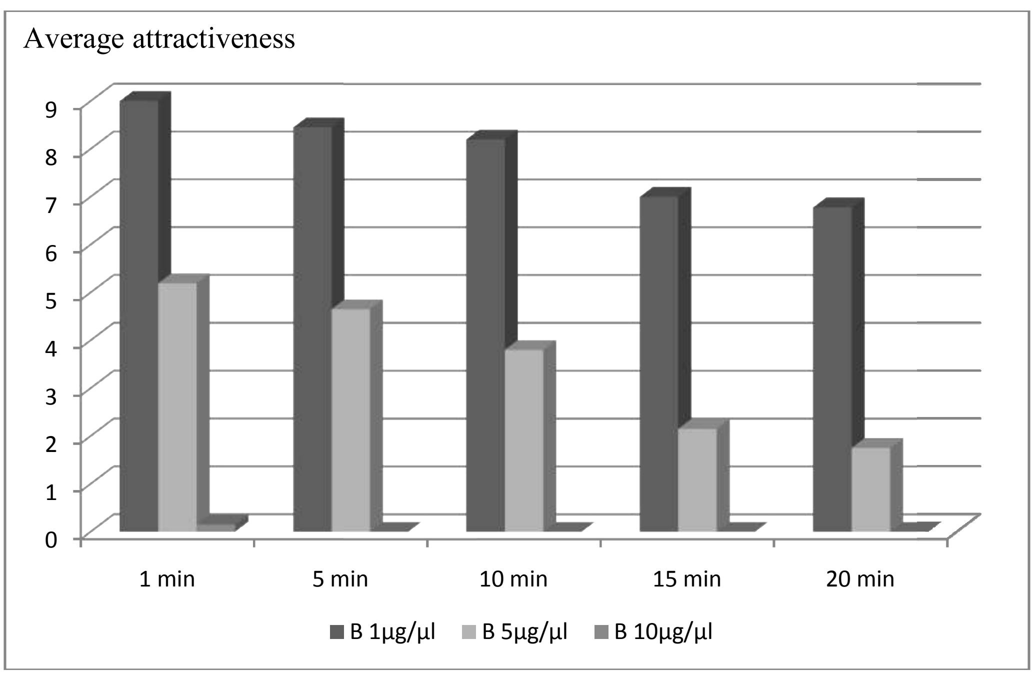

Figure 2: **Attractiveness of neonate larvae by the action of compound B at different doses (1µg/µl, 5µg/µl, and 10µg/µl).**

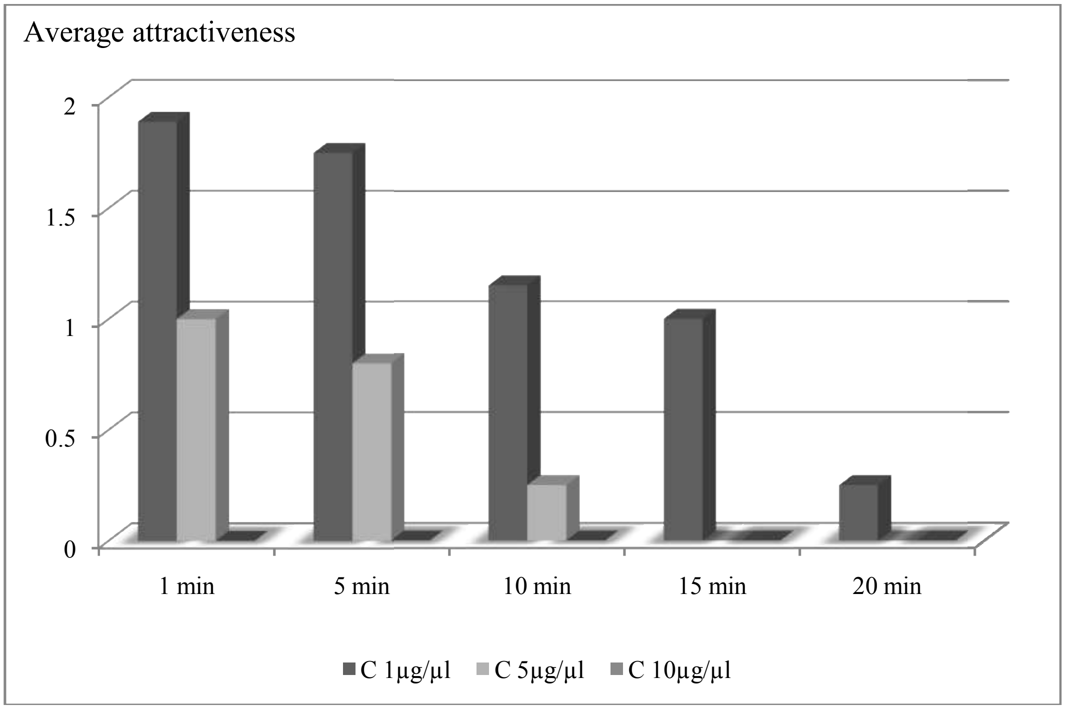

Figure 3: **Attractiveness of neonate larvae by the action of compound C at different doses (1µg/µl, 5µg/µl, and 10µg/µl).**

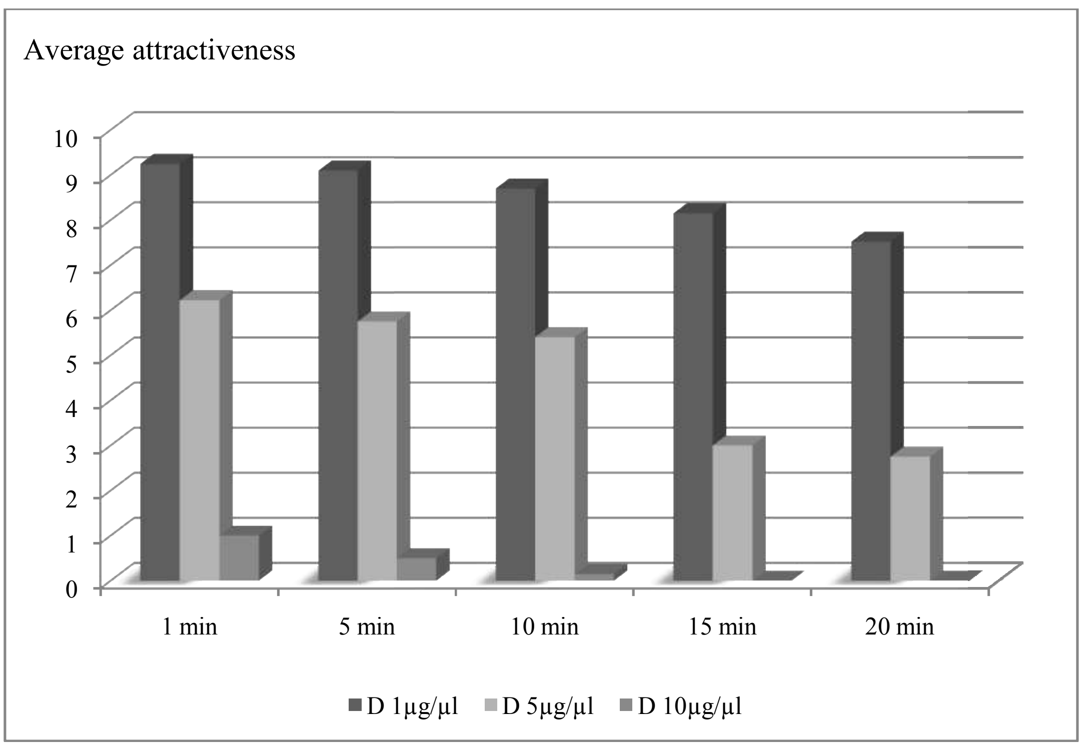

Figure 4: **Attractiveness of neonate larvae by the action of compound D at different doses (1µg/µl, 5µg/µl, and 10µg/µl).**

The average attractiveness of the neonate larvae of C. pomonella apple moth varies according to the mixtures tested. It decreases proportionally with increasing doses used and the experimental period. From the lowest dose of 1µg/µl, the mixture D (β-Caryophyllene, Cis-3-Hexenyl acetate, Farnesene) and the mixture B (β-Caryophyllene, Cis-3-Hexenyl acetate, β-Farnesene) show an attractive effect that is expressed with 9,25and 9 average neonate larvae for the mixture D and for the mixture B respectivelyafter 1minuteof exposition. This average attractiveness decreases proportionally with time, thus, after 5 minutes of exposure we have obtained an average attractiveness equal to 9,1for the mixture D, and 8,45 for the mixture B. The attractive effect of the two mixtures of volatile organic compounds decreases to reach 7,52 for the mixture D, and 6,78 for the mixture B, after 20 minutes of exposure.

At a dose of 5µg/µl, the average attractiveness of the mixtures D andB decreases and expresses6,22 and 5,19average on neonate larvae for the mixture D and for the mixture B respectively after 1 minuteof exposure. This average attractiveness decreases proportionally with time, thus, after 5 minutesof exposure we have obtained an average attractiveness equal to 5,75 for the mixture D, and 4,65 for the mixture B. The attractive effect of the two mixtures of volatile organic compounds decreases to reach 3,75 for the mixture D and 1,75 for the mixture B, after 20 minutes of exposure.

At the highest dose of 10µg/µl, the action of the two mixtures decreases considerably because of the small size of the neonate larvae, which seem very sensitive and try to escape the high concentration odor source. It is expressed with an average of 1 and 0 on neonate larvae for the mixture D and for the mixture B respectively after 1 minute of exposure. This average attractiveness decreases proportionally with time, thus, after 5 minutes of exposure we have obtained an average attractiveness equal to 0,5 for the mixture D, and after 20 minutes of exposure, the attractive effect of the mixture of volatile organic compounds D reaches 0.

The average attractiveness of these two mixtures D andB decreases as doses increase, this can be explained by the relatively small size of neonate larvae that appear to be disoriented and weakened in the presence of volatile organic compounds at high doses.

Mixtures A (β-Caryophyllene, Cis-3-Hexen-1-ol, Farnesene) and C (β -Caryophyllene, Cis-3-Hexen-1-ol, β-Farnesene) express a relatively low attractive effect on neonate larvae of codling moth *C.pomonella* for different concentrations and throughout the duration of exposure.

At the lowest dose of 1µg/µl, mixtures A and C mark a reduced attractive effect expressed with 2,15 and 1,89 average on neonate larvae for the mixture A and for the mixture C respectively after 1 minute of exposure. This average attractiveness decreases proportionally with time, thus, after 5 minutes of exposure the average attractiveness is only 2 for the mixture A and 1,75 for the mixture C. The attractive effect of the two mixtures of volatile organic compounds decreases to reach 0,5 for the mixture A, and 0,25 for the mixture C, after 20 minutes of exposure.

At a dose of 5µg/µl, the average attractiveness of the mixtures A et C decreases and expresses with 1,25 and 1 average on neonate larvae for the mixture A and for the mixture C respectively after 1 minute of exposure. This average attractiveness decreases proportionally with time, thus, after 5 minutes of exposure the average attractiveness is only 1, 1 for the mixture A and 0,8 for the mixture C. After 20 minutes of exposure, the attractive effect of two mixtures of volatile organic compounds greatly decreases to zero.

At the highest dose of 10µg/µl, the action of the two mixtures decreases significantly and does not register any attractiveness because of the reduced size of neonate larvae which seem very sensitive and try to escape the high concentration odor source.

Neonate larvae appear to have a preference between mixtures of volatile organic compounds tested. Thus, they are strongly attracted by the two mixtures D and B which seem to play a highly attractive role.

Mixtures A and C express relatively low attractiveness to neonate larvae that do not seem to appreciate the source of the odor and seek earlier out of the petri dish for the most part. It appears that these two mixtures are involved earlier in a repulsive than attractive effect for the neonate larvae of the codling moth.

Variance analysis with two classification criteria revealed no significant differences for the organic compounds factor (p=0,09), and significant differences for the dose factor (p=0,04). The test of Newman and Keuls at the threshold of 5% highlights two homogeneous groups: A and Bcharacterized by the dose of 1µg/µl and 5µg/µl for the group A and the dose 5µg/µl and 10µg/µl for the group B (Table 1).

Table 1: Newman and Keuls test results regarding the effect of the dose factor.

Labels	**Averages**	**Homogeneous groups**	
Dose 1µg/µl	4,511	A	
Dose 5µg/µl	2,318	A	B
Dose 10µg/µl	0,101		B

4. DISCUSSIONS

The study carried out concerns the behavioral effect of neonate larvae in the presence of different media. The results obtained for the action of the various volatile organic compounds of the different mixtures at different doses with respect to the attractiveness of the larvae of the codling moth of *C. pomonella* are elucidated. The average attractiveness of the neonate larvae of the codling moth varies according to the mixtures tested. It decreases proportionally with the increase of the doses used and the duration of experimentation.

The two mixtures D (β -Caryophyllene, Cis-3-Hexenyl acetate, Farnesene) and B (β -Caryophyllene, Cis-3-Hexenyl acetate, β-Farnesene) recorded a strong appeal for neonate larvae. An attractant is all that is causing decisive stimulus of the insect by a positive response (Dethier, 1947). The average attractiveness of these two mixtures decreases as doses increase, this can be explained by the reduced size of neonate larvae that appear to be disoriented and weakened in the presence of volatile organic compounds at high doses.

The two mixtures A (β -Caryophyllene, Cis-3-Hexen-1-ol, Farnesene) and C (β -Caryophyllene, Cis-3-Hexen-1-ol, β-Farnesene) express relatively low attractiveness to neonate larvae codling moth *C.pomonella*for different concentrations and throughout the duration of exposure. It appears that these two mixtures would participate earlier in a repulsive than attractive effect for neonate larvae of the codling moth *C.pomonella*.

The discovery over 20 years ago of the role of volatile compounds emitted by plants attracting natural enemies has led to a great deal of research on insect plant interactions (Dicke and Sabelis, 1988; Turlings and *al*., 1990; Mumm and Dicke, 2010). All of this research has mobilized different ecological disciplines (behavioral, evolutionary and chemical) and has made a leap forward in the understanding of plant-insect communications.

In the mid-2000s, for the first time, a full-field diffusion of volatile organic compounds (COVs) was attempted to protect vineyards and hop crops by attracting natural enemies (James and Price, 2004).From encouraging results have demonstrated that synthetic VOCs promote the culture of auxiliary attractiveness, research has expanded to different types of agrosystems in recent years (Khan and *al*., 2008; Rodriguez-Saona and *al*., 2011; Simpson and *al*., 2011).

However, the vast majority of studies focusing on the potential of COVs in crop protection have been restricted to biological control. Push-pull strategies represent a new approach in which it is possible to take advantage of the influence of COVson all levels of a tri-trophic system where they act on pest orientation. Gombert and *al*. (2014) evaluated the attractiveness of semio-chemical substances of flowers and cloves in the field on the species *Tychiusaureoles* (Coleoptera: Curculionidae), the results obtained show an attractive effect cloves COV against females of this insect. In the same context, our study makes it possible to evaluate the attractiveness of semio-chemical substances identified at the Versailles INRA in natural conditions with a view to using them to divert the behavior of larvae codling moth. Most chemotaxis is caused by odorants contained in plants (essential oils, volatile compounds), by substances emitted by the insect itself (sexual attraction)or by synthetic chemicals; the chemical substances that determine chemotaxis are perceived by the sensory organs of the olfaction, hence the name chemotropism-olfactory(Chauvin, 1950).

The role of volatile substances in the search for host plants by phytophagous insect larvae varies according to the niches occupied by different larvae in the biosphere (Jones etCoaker 1978).Larvae of some pests can even distinguish odor differences in the same host species (Ascoli and Albert 1985, Landolt and *al*. 2000). For monophages or oligophagous insects underground, some secondary constituents of plants may be specific hosts search indices (Klingler 1958).The majority of above ground feeding insects respond to host-specific volatile plant compounds (Jones and Coaker 1978).For example, first the larvae of the butterfly *Heliothisarmigera* responded to terpenes (Rembold and*al*., 1989); neonate larvae of codling moth, *Cydia pomonella* (L.) answered to l'α-farnésène (Sutherland and Hutchins, 1972,1973; Bradley and Suckling 1995; Witzgall and *al*., 2005), and alkyl esters and ethylic and decadienoic acid (Knight and Light 2001).Casadoand *al*. (2006) performed analyzes on male antennae of codling moth *C. pomonella* such as sensors using volatile substances taken from almost ripe apples, they identified six compounds that caused the activity electro-antennography, namely: hexyl acetate, 4,8-dimethyl-1,3,7-nonatriene, (Z) -3-hexenol, nonanal, hexyl butylate, (E, E) -α-farnesene and 2-cyclopentyl cyclopentanone. Ansebo and *al*. (2005) have identified olfactory receptor neurons in the antennae of the female codling moth *C. pomonella*for the pear ester, namely: (E, E) -α-farnesene and (E) -4,8-dimethyl-1,3,7-nonatriene. Coracini and *al*. (2004) reported the attraction of males of C. pomonella codling moth to volatile mixtures containing both 1,3,7-nonatriene and (E, E) -α-farnesene. Knight and *al*. (2011) reported the attraction of female codling moth to 4,8-dimethyl-1,3,7-nonatriene.

Pests use volatile plant compounds as an important signal to detect the location of their host (Dicke, 2000). The apple and pear trees are the main host plants of the codling moth *C. pomonella*, the larvae can adapt and develop also on walnut and quince, causing significant economic damage that is not insignificant, several studies have been conducted to identify volatile organic compounds of apple involved in the attraction of codling moth (Bäckman and *al*., 2001, Light and *al*., 2001;Ansebo and *al*., 2005; Light and Knight, 2005).

Most studies focusing on the potential of synthetic COVs in crop protection have focused for practical reasons on the diffusion of isolated compounds. However, the few authors who have studied the possibility of diffusing COV mixtures suggest that such assemblages modify the behavior of insects more effectively. Indeed, Tóth and *al*., 2009 have for example noticed that the addition of methyl salicylate makes possible to increase the attractiveness of a mixture of two COVs for *Chrysoperlacarnea* while this chrysopus does not respond to methyl salicylate when isolated. An interesting approach in the assembly of attractive COVs has been proposed by Del Soccoro and *al*., 2010 who studied the possibility of modifying the behavior of a pest from a mixture of volatile compounds emitted by host plants and non-host plants used by the insect to feed themselves.

The combination of COVs to obtain a mixture that tries to recreate odors emitted by host plants has several advantages. The response of an insect to an olfactory stimulus depends on the environmental context: learning phenomena allow an insect (whether phytophagous or entomophagous) to preferentially respond to the olfactory signals emitted by an abundant host in the environment rather than the olfactory signals emitted by a host that is not present at the local level (Webster and *al*., 2008).

A good mix might be less sensitive to these learning behaviors and would be effective in contrasting environments. In addition, a mixture of COVs limiting learning phenomena would also be useful in a context of sustainability of strategies based on the behavioral modification of insects. However, it is important to note that determining the relative proportions of COVs to be included in a mixture is often problematic (Kaplan, 2012). This type of strategy integrates the two main principles on which agro-ecology is based: Increasing biodiversity and strengthening biological regulations (Wezel and *al.*, 2009).

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