Preliminary results of foliar applications of fructose to reduce codling moth *Cydia pomonella* L. (Lepidoptera, Tortricidae) damages on apple tree in organic farming

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Abstract

*In precedent field trials, it has been observed that the application of low dose of sucrose significantly reduced the means of infested fruits by the codling moth. The mean Abbott efficacy of sucrose was 41.0±10.0 % but was lower than the CpGV (*Cydia pomonella *granulovirusis) one. The effects of an other sugar, fructose, was also investigated to reduce the damages of the codling moth. We tested applications of low doses of fructose (0.01 %) in four commercial orchards from 2013 to 2014 in Algeria and in France. The activities of fructose were assessed by comparison with untreated controls, trees treated with the CpGV and trees treated with sucrose (0.01 %). Foliar applications of micro-doses of fructose (0.01 %) every twenty days in commercial orchards protected as much as the CpGV against the codling moth. This work opens a route for the development of strategies using the new concept of "sweet immunity" inducing and for the use of new traits for plant breeding.*

Keywords: *Cydia pomonella* granulovirusis, immunity, sugars, defence inducer

Introduction

The plant defence inducing can be considered as a new challenge in organic farming. The priming is defined as increased readiness of defence induction. Priming has often been considered in the context of plant pathogen interactions but plants can also be primed by signals associated with herbivore feeding (Frost *et al*., 2008).

For a long time it was found that injured plants have their sugar levels increased, known as "high sugar resistance"[19]. The role of sugars signalling in plant defense responses against fungal pathogens are more and more studied and discussed in literature (Trouvelot, 2014). Oligosaccharides are widely accepted as players in plant innate immunity (Bolouri Moghaddam & Van Den Ende, 2012) but knowledge has led to the new concept of "sweet immunity" and "sugar-enhanced defense" in which saccharides-likes should also play an important role in such process as well as tolerance to stress. Sugars could act as "priming" molecules inducing preparation of the plant to defend itself more quickly and intensely in case of micro-organisms attacks or stress. The mechanisms involved in "sweet immunity" are still ongoing research but it seems that response of plant innate immunity to pathogens through sugar-signalling and hormonal pathways greatly could depend on the actual status of circadian clock (Bolouri Moghaddam & Van Den Ende, 2013).

Very few studies have been carried on primary metabolites at the leaf surface that act as signals on the Lepidoptera females upon recognition of the plant site to lay their eggs. The studies (Lombarkia *et al*., 2002, 2008) show the important role of sugars on *C. pomonella*. Egg-laying site preference within the apple tree and its intensity are related to a blend of sucrose, D-fructose, glucose, sorbitol, quebraquitol, *myo*-inositol present at the surface of the apple tree. The exogenous foliar application of sucrose and D-fructose can induce resistance by antixenosis to the insect egg-laying (Lombarkia *et al*., 2008). This concept of

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exogenous application of sugars every 20 days on apple tree to modify the egg-laying of *C. pomonella* to reduce its damages was tested in commercial orchards of several countries and on several years (Derridj, 2012; Arnault *et al*., in press). The application of sucrose at 0.01 % reduced the means of infested fruits. The mean Abbott efficacy of sucrose was 41.0 ± 10.0 %. When we compared the effects of sucrose 0.01 % and CpVG (*Cydia pomonella* granulovirusis) in organic farming, the mean of damages of the sucrose treatment had intermediate level of fruit damages (17.6 ± 3.7 %) between untreated modality and CpVG modality. The CpGV reduced significantly the codling moth fruits damages contrary to the sucrose application (Arnault *et al*., in press Pest Management Science).

The results about the role of sugars on the egg-laying of *C. pomonella*, the efficacy of sucrose application in apple orchards and the new concept of “sweet immunity” still under investigation are challenges for exploiting sugars to reduce susceptibility of plants to bio-pests. Studies reported here determine the comparative efficacy of fructose, sucrose and CpVG in micro-dose against fruits damages due to *C. pomonell*a. Orchard tests were conducted during two years. The potential of using this method to develop an effective control of codling moth in strategies of protecting the apple tree in orchards in organic farming and in integrated fruit production is discussed.

Material and Methods

Seven experiments were conducted in apple orchards in Algeria and France from 2013 to 2014. Several treatment controls on *C. pomonella* were applied on apple trees (*Malus domestica* Borkh.) in organic farming. Each bioassay was randomised in block. Plots were arranged in a randomized Fisher block design. In all trials in several countries over several years, the variable ‘% of infested fruit at harvest’ is based on the ratio of the total number of infested fruits (fallen and damaged) and the total number of infested fruits (fallen and damaged) per plot. Each block (groups of experimental units) in France and Algeria was as homogeneous as possible in equal numbers of treatment modes. With each block, the treatments were distributed randomly and independently of their distribution in other blocks. The number of trees was calculated to be able to observe a minimum of 250 fruits in each elementary plot harvest (250 to 530 fruits).

The first application of sugar at 0.01 % (0.1 $g.l^{-1}$) took place 20 days before the maximum egg-laying period of the second generation and was renewed within a 20-day interval until the harvest. Sugar spray solutions was applied between 7:30 a.m. and 9:30 a.m. Fructose (CAS Number 57-48-7) and sucrose (CAS Number 57-50-1) were purchased from Sigma-Aldrich (St. Louis, MI, USA).

Five studies in Algeria were conducted in four localities in the region of Batna in the dominant climate of steppe. The apple orchards were composed of the apple cultivars Anna, Golden Delicious and Starkrimson. These trials were conducted on the three generations of *C. pomonella*. Three treatments modes were compared in similarly organic farming: (i) fructose at 0.01 % and (ii) the product Madex® alone (isolat GV-0006).

Two field trials in France were conducted in commercial orchard located in one locality in the region of Avignon. They took place in Mediterranean climate that is warm temperate. The apple orchards were composed of the apple cultivars Gala. These trials were conducted on the two generations of *C. pomonella*.

Two treatments modes were compared in organic farming production: (i) fructose at 0.01 %; (ii) Carpovirusine®Evo2 (isolat NPP-R5) in first generation larvae applied every 20 days and Carpovirusine^{TM}2000 (isolat CpGV-M) applied every 20 days in second generation larvae.

The means between each field trials were compared by a non parametric Kruskal-Wallis ANOVA of ranks tests followed by post-hoc analysis with Fisher and Turkey's test. A P-value of 0.05 was used to establish significance in all tests. All analyses are performed using XLSTAT software version 2012.2.02 (Addinsoft).

Results

The mean of percentage of infested fruits of the CpGV and fructose modalities (10.8 ± 2.8 % and 8.1 ± 2.8 % respectively) were significant lower than the untreated modality (23.8 ± 4.6 %) (Fig.1). However, the results were not statistically different for sucrose treatment. Actually, the mean of damages of the sucrose treatment had intermediate level of fruit damages (17.6 ± 3.7 %) between the untreated modality and the CpVG and fructose modalities. The CpGV and fructose treatment reduced significantly the codling moth fruits damages contrary to the sucrose application. The mean of the efficacy Abbott for fructose 0.01 % and CpGV were 48.9 ± 9.1 % and 40.8 ± 10.2 % respectively that were slightly more than the sucrose one (40.8 ± 11.1 %). We concluded that fructose applications (0.01 %) were as effective as the CpVG.

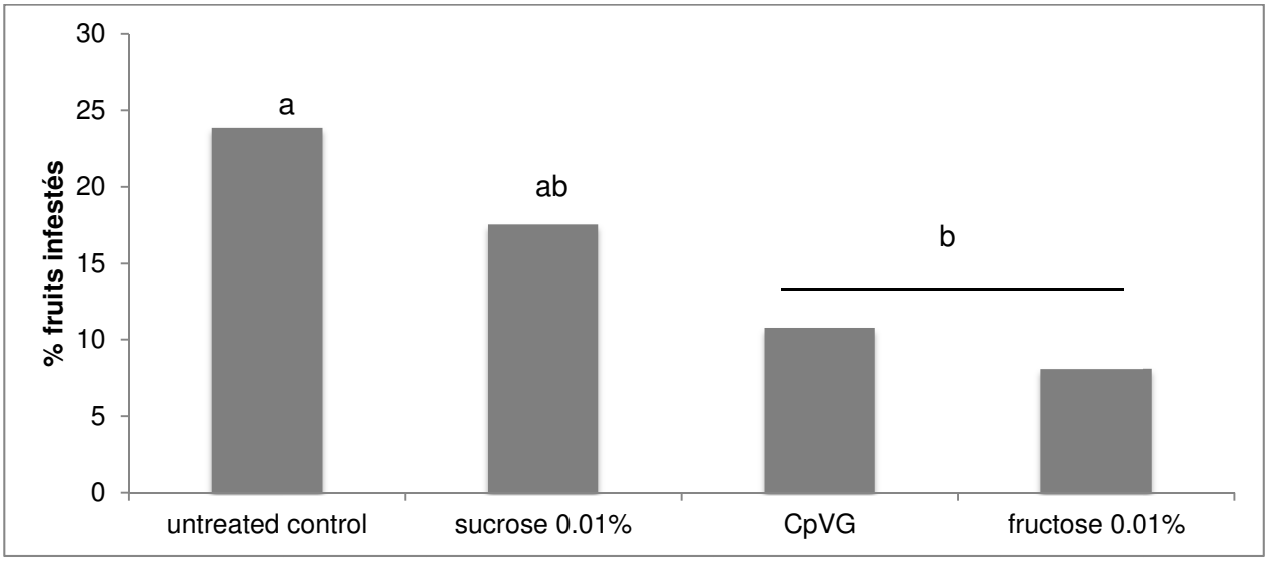

Figure 1: Comparison of the means of the percentage of infested fruits in apple orchards in Algeria and in France (n = 7). Control treatments of *C. pomonella* are as follows : foliar applications of sucrose and fructose at 0.01 %, *Cydia pomonella* granulosis virus (CpGV) and the untreated modality with no product applied. Data in columns with different letters are statistically different according to Fisher's test at P = 0.05.

Discussion

The strategy of foliar applications of sugars solution is rather simple, it consists in applications in the morning before 7 a.m. (solar time) just after its preparation, every 20 days. This work opens a door to development of new strategies applying the new concept of "sweet immunity" and to consideration of new selection traits for plant breeding. In this work, no related evidence on the results and the sweet immunity concept was provided. But it should stimulate research on "sweet immunity" network for the development of new strategies in a context of sustainable crop production. The experimental designs could be improved with a foliar application by water that represents the best control treatment in our conditions. Indeed, since such a control treatment (i.e. foliar spray of water) could be perceived by the plant as an abiotic stress and could induced some defence reactions in

the plant, it is thereby not obvious to make a difference between the plant reactions induced in response to sucrose to those induced in response to foliar spray. Moreover, EU has approved fructose in subdoses in august 2015 (No 1492/2015) as a basic substance for plant protection purpose (apple trees orchards against *C. pomonella*, and maize against *Ostrinia nubilalis* at the vegetative stage).

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