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Foliar application of microdoses of sucrose to reduce codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) damage to apple trees

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Abstract

BACKGROUND: The effects of foliar applications of microdoses of sucrose to reduce the damage by the codling moth have been reported from nine trials carried in France and Algeria from 2009 to 2014. The activity of sucrose alone was assessed by comparison with an untreated control and some treatments with the *Cydia pomonella* granulovirus or a chemical insecticide. The addition of sucrose to these different treatments was also investigated.

RESULTS: The application of sucrose at 0.01% reduced the means of infested fruits with a value of Abbott's efficacy of 41.0 ± 10.0%. This involved the induction of resistance by antixenosis to insect egg laying. Indeed, it seems that acceptance of egg laying on leaves treated with sucrose was reduced. The addition of sucrose to thiacloprid improved its efficacy (59.5% ± 12.8) by 18.4%. However, the sucrose had no added value when associated with *C. pomonella* granulovirus treatments.

CONCLUSION: Foliar applications of microdoses of sucrose every 20 days in commercial orchards can partially protect against the codling moth. Its addition to thiacloprid increases the efficacy in integrated control strategies, contrary to *C. pomonella* granulovirus treatments. This work opens a route for the development of new biocontrol strategies.

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Keywords: *Malus domestica* Borkh; orchards; sweet immunity; field experiment, Abbott's efficacy

1 INTRODUCTION

Cydia pomonella L. (Lepidopetera: Tortricidae) is regarded as one of the most damaging insect pests in apple orchards worldwide.[1] Larvae bore into fruits and feed on the core, causing damage. Tolerance levels of *C. pomonella* infestations in apple production are extremely low, with infested apples being unmarketable. The extensive use of chemical insecticides has, however, resulted in the development of insecticide-resistant field populations of *C. pomonella*, with resistance against various active ingredients.[2] Biological control of *C. pomonella* has become a solution to counteract the increase in insecticide resistance of codling moth populations and to minimise the use of chemical insecticides. The application of *C. pomonella* granulovirus (CpGV) and pheromone-based mating disruption are the most commonly used control measures of *C. pomonella* in organic production. CpGV is an extremely specific and highly virulent pathogen of *C. pomonella* and has comparable efficacy to many chemical insecticides.[3,4] CpGV was first based on the Mexican isolate CpGV-M (product Madex® and Carpovirusine™ 2000). Since 2004, local *C. pomonella* have been reported to show a reduced susceptibility to CpGV-M in some organic orchards in Germany and France,[5] and this resistance has now extended to other European countries. In order to bypass this resistance occurring by repetitive applications of CpGV, research was conducted to obtain new viral variants able to control these resistant insect populations. Various natural isolates were found to be able partially to overcome the resistance, such as NPP-R5 (Carpovirusine® Evo2, registered in 2012).

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In the current evolution of pesticide reduction, the development of new biocontrol strategies is necessary. Plant defence induction can be considered as a new challenge in agriculture. 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.[6] Research on priming of antiherbivore attack has been focused on herbivore-inducible plant volatiles (HIPVs) that are produced and released by neighbouring plants (or plant parts) under herbivore attack.[7] Different mechanisms of defence other than HIPVs have been reported, including priming of defence by insect oviposition.[8]

Very few studies have been carried out on primary metabolites at the leaf surface that act as signals to lepidopteran females upon recognition of the plant site to lay their eggs. Most leaf sugars (glucose, fructose, sucrose) are thought to originate as photosynthates from the plant interior and to end up on the surface by passive diffusion in a process called leaching.[9] Their quantities at the leaf surface follow a dynamic equilibrium between the inside and outside of the plant and are influenced by epiphytic microorganisms.[10] Although it remains to define precisely the role of phyllosphere microflora on the bioavailability of leaf sugars, several models predict the diffusion of sugars and other plant metabolites from the apoplast across the leaf cuticule and their consumption by epiphytic microorganisms.[11] The studies[12,13] show the important role of primary metabolites and particularly sugars on *C. pomonella*. Egg laying site preference within the apple tree and its intensity are related to a blend of three soluble carbohydrates (sucrose, D-fructose and glucose) and three sugar alcohols (sorbitol, quebraquitol, *myo*-inositol) present at the surface of the apple tree. The quantities and proportions of the blend compounds can vary with the organ, stage of development and variety. The exogenous foliar application of sucrose and D-fructose can induce resistance by antixenosis to insect egg laying.[13] Actually, these foliar applications of microdoses of sucrose (10^{-3}%) or D-fructose (10^{-4}%) induced changes in the composition of the water-soluble metabolome at the bourse shoot leaf surfaces that are usually preferred for egg laying. The modified 'blend signal' composition reduces egg laying in the laboratory. This concept of exogenous application of sugars every 20 days on apple trees to modify the egg laying of *C. pomonella* to reduce the damage it causes was tested in commercial orchards of several countries and on several years.[14] The methods enabled a significant reduction in damage with Abbott's efficacies of 40.6 ± 8.4% and 37.2 ± 11.0%, respectively, for D-fructose 0.01% and sucrose 0.001%, the efficacies of which were similar and not significantly different. This paper describes new experiments with this alternative method, and the sugars can be envisaged as non-HIPV-mediated priming of defence.

It has long been known that injured plants have increased sugar levels, known as 'high sugar resistance'.[15] Defence responses are tightly linked to the upregulation of sink metabolism to satisfy the energy requirements of the activation of the cascade of defence reactions.[16] The role of sugar signalling in plant defence responses against fungal pathogens being increasingly studied and discussed in the literature.[17] Increasing interest is being shown in the relationships between sugars and plant immunity and defence/resistance to biotic and abiotic stresses.[18–20] Oligosaccharides are widely accepted as players in plant innate immunity, but knowledge has led to the new concept of 'sweet immunity' and 'sugar-enhanced defence', in which saccharide analogues should also play an important role in such processes[21,22] as well as tolerance to stress.[23] Sugars could act as 'priming' molecules inducing preparation of the plant to defend itself more quickly and intensely in the case of microorganism attack or stress.[24,25] The mechanisms involved in 'sweet immunity' have not been elucidated, but it seems that the response of plant innate immunity to pathogens through sugar signalling and hormonal pathways could depend greatly on the actual status of the circadian clock. In *Arabidopsis* cell suspensions, sucrose or glucose induces the expression of several pathogen-related (PR) genes through a salicylic acid (SA)-dependent pathway.[26] Exogenous applications of sucrose in minor wounds change its concentration in the apoplast and plant cells and thus constitute signals stimulating jasmonic-acid-dependent defence pathways.[27] Sugars may function as extracellular indicators of pathogen infection.[27] Sucrose induces isoflavonoid synthesis as a defence response against *Fusarium oxysporum* in lupine.[28] There is a highly sophisticated network between different signal transduction pathways, including reactions to biotic and abiotic stresses, hormone and reactive oxygen species (ROS) signalling, growth and plant development and metabolic regulation, the whole interacting with diurnal and circadian rhythms.[22,29–34] Moreover, a key functional link between the circadian clock and plant immunity was shown for the first time in *Arabidopsis* mutants.[35]

Thousands of plant genes are sugar responsive. Sucrose, D-fructose and D-glucose regulate 209 genes divided into seven subgroups, representing their responsiveness to a specific set of sugars; only ten genes are common to the three sugars.[36] Each sugar should be perceived by separate specific sensing mechanisms. Research is continuing to help understand how sugars are specifically transported[37–40] and sensed by plants. Glucose and sucrose transporters are located at the plasma membrane. The first fructose transporter was described recently in *A. thaliana* leaves. The carrier protein SWEET17 is localised at the vacuolar membrane and can act as an importer and exporter of fructose.[41] Hexokinase enzymes act as glucose sensors,[42–47] and sucrose should be sensed at the level of the plasma membrane or by an intracellular sensor.[44,48,49] Then, hydrolysed in the apoplast by invertases in hexoses (glucose and fructose), the sucrose/hexose ratio should be perceived by membrane or cytosolic sensors.[17,21] Yeast invertase in the apoplast of tobacco induces the production of PR proteins and increases resistance against virus infection in tobacco.[50]

The progressive discovery of the multiple roles of sugars in defensive and resistance systems of the plant leads to their exploration for use in plant protection management against their biotic and abiotic environment.[51,52] Their safety for the environment and organisms make the task even more attractive. In most cases the polysaccharides (glucan laminarin, chitosan, etc.) have been tested individually[53] and in combination[54] against pathogens. Their limited current use in crop protection may be attributed (i) to discrepancy between the efficacies observed for a single stress in the laboratory and for the multitude of stresses in the field, (ii) to difficulties in separating antimicrobial activities from defence/resistance elicited in plants and the tissue regeneration induced, (iii) to varietal selection, which might lower defence capacities and/or innate immunity, (iv) to difficulties in finding molecules and formulations to make sugars at the leaf surface that are less susceptible to external factors before passing through the cuticle and (v) to competitiveness with plant protection products still currently authorised in terms of their effectiveness.

Results concerning the role of sugars in the egg laying of *C. pomonella*,[12,13] the efficacy of sugar application in apple

Table 1. Comparison of the percentage of infested fruits in apple orchards ($n = 9$) in organic farming (OF) or in integrated pest management (IPM). Control treatments of *C. pomonella* were as follows: foliar application of sucrose 0.01% according to Section 2.1.1, *C. pomonella* granulosis virus (CpGV), CpVG with the addition of sucrose 0.01%, chemical insecticide (thiacloprid), chemical insecticide (thiacloprid) with the addition of sucrose 0.01% and the untreated modality with no product applied[a]

Year/country/cultivar/IPM or OF	Untreated control	Sucrose 0.01%	CpVG	CpVG + sucrose 0.01%	Chemical insecticide	Chemical insecticide + sucrose 0.01%
2009/Algeria/'Anna'/IPM	29.9(±0.5) a	26.0(±0.2) b	–	–	22.0(±1.0) b	15.6(±2.2) b
2010/Algeria/'Golden Delicious'/IPM	38.6(±6.0) a	10.5(±0.4) b	–	–	3.4(±0.6) c	1.8(±0.9) d
2011/Algeria/'Golden Delicious'/IPM	46.8(±2.8) a	15.2(±0.2) b	–	–	8.5(±0.4) c	4.5(±0.4) d
2013/France/'Granny Smith'/IPM	12.0(±1.5) a	–	–	–	2.5(±2.5) b	4.1(±0.8) b
2013/Algeria/'Anna'/OF	34.3(±3.0) a	6.6(±1.3) b	7.4(±0.7) c	–	22.4(±0.99) b	–
2013/France/'Gala'/OF	14.9(±2.5) a	13.5(±3.9) ab	8.8(±2.0) ab	5.1(±2.8) ab	–	–
2014/Algeria/'Starkrimson'/OF	32.4(±1.4) a	28.3(±1.4) b	25.8(±1.0) b	19.9(±1.0) c	–	–
2014/Algeria/'Royal Gala'/OF	26.5(±2.4) a	17.5(±0.8) b	11.4(±2.5) c	10.3(±1.9) c	–	–
2014/Algeria/'Anna'/OF	37.3(±3.0) a	22.0(±3.1) b	12.3(±1.5) c	11.2(±0.7) c	–	–

[a] Within a line, means followed by a different letter are significantly different according to the Student–Newman–Keuls test, $P = 0.05$. (±SE): standard error value A dash (—) denotes no experimental data.

orchards[14] and the new concept of 'sweet immunity'[21,22] still under investigation present challenges in exploiting sugars to reduce the susceptibility of plants to biopests. Our studies determined the efficacy of the foliar application of sucrose in microdoses against fruit damage due to *C. pomonella*. Orchard tests were conducted over a 5 year period with sucrose applied with or without CpGV or chemical insecticide. The effectiveness of these seasonal programmes against codling moth and their integration in apple production are reported. The potential of using this method to develop an effective codling moth control in strategies for protecting apple trees in orchards in organic farming and in integrated fruit production is discussed.

2 EXPERIMENTAL METHODS

2.1 Field experiments

Nine experiments were conducted in apple orchards in Algeria and France from 2009 to 2014. Several treatment controls on *C. pomonella* were applied on apple trees (*Malus domestica* Borkh.) under agricultural production with specification of production conditions, e.g. integrated apple production and organic farming (Table 1). Cultivars tested in this work all exhibit a susceptibility to codling moth. The level of sensibility is difficult to ascertain because it depends on years, on the climate and on the populations of *C. pomonella*. Each bioassay was randomised in block. Plots were arranged in a randomised Fisher block design. In all trials in several countries over several years, the variable 'percentage of infested fruits 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. Abbott's formula[55] is very commonly used in field trials. Its efficacy at harvest measures the percentage of *C. pomonella*-infested fruits versus untreated controls according to the formula

$$\frac{T_0 - T_t}{T_0} \times 100$$

where T_0 is the percentage of infested fruits in the untreated plots and T_t is the percentage of infected fruits in the treated plots.

2.1.1 *Foliar applications of sucrose at 0.01%*

The first application of sucrose 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 harvest. Sugars and insecticide spray solutions were applied between 7:30 a.m. and 9:30 a.m. Sucrose (CAS number 57-50-1) was purchased from Sigma-Aldrich (St Louis, MO).

2.1.2 *Studies in Algeria*

Seven studies in Algeria were conducted in a commercial orchard managed similarly to organic farming requirements allocated in the region of Batna in the dominant steppe climate. The apple orchards consisted of 4–6 rows, with 12–18 trees per row of the apple cultivars 'Anna', 'Golden Delicious', 'Strakrimson' and 'Royal Gala'. These trials were conducted on three generations of *C. pomonella*.

Three treatment modes were compared in organic farming production: (i) sucrose at 0.01% as described in Section 2.1.1; (ii) the product Madex® alone (isolat GV-0006); (iii) sucrose 0.01% combined with the Madex® product. The sucrose in (i) and (iii) was applied 7 times as described in Section 2.1.1, 20 days apart, in the morning.

Three treatment modes were compared in chemical production: (i) sucrose at 0.01% as described in Section 2.1.1; (ii) thiacloprid; (iii) sucrose 0.01% added to thiacloprid. The sucrose in (i) and (iii) was applied 6 times as described in Section 2.1.1, 20 days apart, in the morning. Thiacloprid was used at the registered dose (0.25 L ha^{-1}, 2 times), and Madex® was used at the doses recommended (0.1 L ha^{-1}, every 8 days from caterpillars of the first generation).

Each block (group of experimental units) was as homogeneous as possible in equal numbers of treatment modes. Four replicates of each modality (treatment) were included in the experimental design. The number of trees was calculated to be able to observe a minimum of 250 fruits in each elementary plot harvest (250–530 fruits).

Applications were conducted with a towed jet sprayer using a spray volume of 1000 L ha^{-1} to ensure effective wetting of the vegetation according to good agricultural practice. The quantities were 0.1 L ha^{-1} for the Madex® and 0.1 g L^{-1} for sucrose (0.01%).

Table 2. Comparison of the means of Abbott's efficacy in apple orchards ($n = 9$) in organic farming (OF) or in integrated pest management (IPM). Control treatments of *C. pomonella* were as follows: foliar application of sucrose 0.01% according to Section 2.1.1, *C. pomonella* granulosis virus (CpGV), CpVG with the addition of sucrose 0.01%, chemical insecticide (thiacloprid) and chemical insecticide (thiacloprid) with the addition of sucrose 0.01%

Year/country/cultivar/IPM or OF	Sucrose 0.01%	CpVG	CpVG + sucrose 0.01%	Chemical insecticide	Chemical insecticide + sucrose 0.01%
2009/Algeria/'Anna'/IPM	13.6	–	–	25.9	47.7
2010/Algeria/'Golden Delicious'/IPM	71.8	–	–	90.2	94.5
2011/Algeria/'Golden Delicious'/IPM	67.3	–	–	81.7	90.3
2013/France/'Granny Smith'/IPM	–	–	–	65.8	79.1
2013/Algeria/'Anna'/IPM	80.8	78.4	–	33.7	–
2013/France/'Gala'/OF	9.5	40.6	65.8	–	–
2014/Algeria/'Starkrimson'/OF	11.9	19.6	34.0	–	–
2014/Algeria/'Royal Gala'/OF	31.7	54.0	58.7	–	–
2014/Algeria/'Anna'/OF	41.2	66.2	69.5	–	–
Mean	41.0(±10.3) b	51.8(±10.2) ab	57.0(±8) ab	59.5(±12.8) ab	77.9(±10.6) a

[a] Within a line, means followed by a different letter are significantly different according to Fisher's test at $P = 0.05$. (±SE): standard error value. A dash (—) denotes no experimental data.

2.1.3 *Studies in France*

Two field trials in France were conducted in commercial orchards managed according to organic farming requirements and located in four localities in the region of Avignon. They took place in a warm, temperate Mediterranean climate. The apple orchards consisted of four rows, with 12–18 trees per row of the apple cultivars 'Granny Smith' and 'Gala'. These trials were conducted on two generations of *C. pomonella*.

Three treatment modes were compared in organic farming production: (i) sucrose at 0.01%; (ii) Carpovirusine® Evo2 (isolat NPP-R5) in first-generation larvae, applied every 20 days, and Carpovirusine™ 2000 (isolat CpGV-M), applied every 20 days in second-generation larvae; (iii) sucrose 0.01% added to Carpovirusine® Evo2 in first-generation larvae, and sucrose 0.01% added to Carpovirusine™ 2000 in second-generation larvae. Carpovirusine® Evo2 is more effective than Carpovirusine™ 2000 on *C. pomonella* populations resistant to CpGV-M isolate. Carpovirusine® Evo2 is an effective solution to resistance when alternated with Carpovirusine™ 2000. The sucrose in modalities (i) and (iii) was applied 5 times as described in Section 2.1.1, 20 days apart, in the morning. Carpovirusine™ 2000 and Carpovirusine® Evo2 were used at the doses recommended (1 L ha^{-1}, every 8 days from caterpillars of the first generation).

Three treatment modes were compared in one field trial in integrated fruit production: (i) sucrose at 0.01% as described in Section 2.1.1; (ii) thiacloprid; (iii) sucrose 0.01% added to thiacloprid. The sucrose in modalities (i) and (iii) was applied 7 times as described in Section 2.1.1, 20 days apart, in the morning. Each block (group of experimental units) was as homogeneous as possible in equal numbers of treatment modes. Four replicates of each modality (treatment) were included in the experimental design. The number of trees was calculated to be able to observe a minimum of 250 fruits in each elementary plot harvest.

Applications were conducted with a mechanical jet sprayer using a spray volume of 1000 L ha^{-1} to ensure effective wetting of the vegetation according to good agricultural practice. The quantities were 0.1 L ha^{-1} for the Madex® and 0.1 g L^{-1} for sucrose. Thiacloprid was used at the registered dose (0.25 L ha^{-1}, 2 times).

2.2 Statistical analysis

The percentages of infested fruits were separately analysed in each trial by ANOVA, followed by *post hoc* analysis using the Student–Newman–Keul test (Table 1). The means between each field trial were compared by a non-parametric Kruskal–Wallis ANOVA on ranks test, followed by *post hoc* analysis using Fisher's and Tukey's tests (Table 2 and Figs 1 to 3). A P-value of 0.05 was used to establish significance in all tests. Comparison of the percentages of infested fruits in the modality of sucrose application at 0.01% and the untreated orchard was done by the Mann–Whitney test at $P < 0.05$ (Fig. 4). All analyses were performed using XLSTAT software v.2012.2.02 (Addinsoft, Brooklyn, NY).

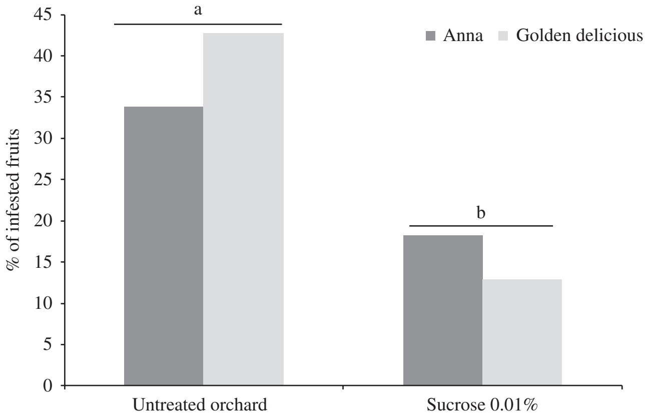

Figure 1. Comparison of the means of the percentage of infested fruits in apple orchards in Algeria ($n = 3$) for 'Anna' and 'Golden Delicious' cultivars. Control treatments of *C. pomonella* were as follows: foliar application of sucrose 0.01% according to Section 2.1.1 and the untreated control with no product applied. Data in columns with different letters are statistically different according to Fisher's test at $P = 0.05$. Bars represent standard errors.

3 RESULTS

3.1 Effects of sucrose 0.01% alone on codling moth fruit damage in orchards

The data of the nine orchard trials since 2009 were analysed. Table 3 shows the percentages of infested fruits with the different control treatments applied against *C. pomonella*. There were significant differences in the fruit damage occurring among treatments in each field trial. The infestation of the untreated control varied from 12 to 46.8%, which represented a very high

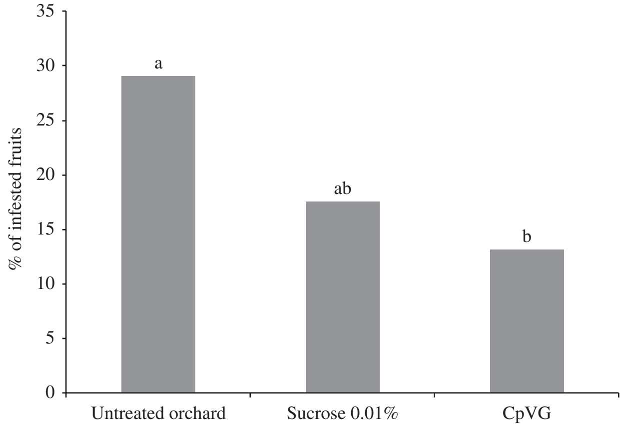

Figure 2. Comparison of the means of the percentage of infested fruits in apple orchards in Algeria and in France (*n* = 5). Control treatments of *C. pomonella* were as follows: foliar application of sucrose 0.01% according to Section 2.1.1, *C. pomonella* granulosis virus (CpGV) and the untreated control with no product applied. Data in columns with different letters are statistically different according to Fisher's test at *P* = 0.05. Bars represent standard errors.

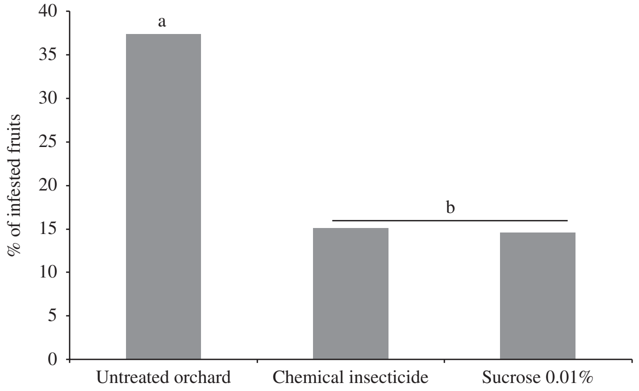

Figure 3. Comparison of the means of the percentage of infested fruits in apple orchards in Algeria and in France (*n* = 4). Control treatments of *C. pomonella* were as follows: foliar application of sucrose 0.01% according to Section 2.1.1, chemical insecticide (thiacloprid) and the untreated control with no product applied. Data in columns with different letters are statistically different according to Tukey's test at *P* = 0.05.

pressure of the pest. Each application of sucrose alone reduced significantly the percentages of infested fruits, except for the field trial in France on the 'Gala' cultivar, i.e. 87.5% of the field trials (Table 1). The mean percentage of damaged fruits with the sucrose treatment was 17.5 ± 2.7%, significantly lower than the untreated modality (32.6 ± 3.3%) (Fig. 4). This result showed the efficacy of the single sucrose treatment, as described in Section 2.1.1, against *C. pomonella* fruit damage. The mean Abbott's efficacy (Table 2) for the sucrose 0.01% treatment was 41.0 ± 10.0%. As shown in Fig. 1, treatment with sucrose 0.01% on both cultivars 'Anna' and 'Golden Delicious' gave similar results. We concluded that there was no varietal effect of the sucrose treatment on these cultivars used for the interpretation of the data. In any case, variations in efficacy between years are more important than those observed between varieties.

3.2 Effects of sucrose 0.01% on codling moth fruit damage in organic farming using CpVG

The mean percentage of infested fruits of the CpGV treatment (13.6 ± 3.3%) was significantly lower than the untreated control (29.5 ± 2.7%) (Fig. 2). The percentage of infested fruits of the CpGV + sucrose treatment was significantly lower than in the untreated control (Table 1), except for the field trial with Carpovirusine™ 2000 + Carpovirusine® Evo2 (France, cultivar Gala) where populations of *C. pomonella* were identified as resistant to the CpVG. However, the results were not statistically different for the sucrose treatment. Actually, the mean damage in the sucrose treatment had an intermediate level of fruit damage (17.6 ± 3.7%) between the untreated control and the CpVG

Table 3. Description of the field trials (n = 9) with control treatments of *C. pomonella* in organic farming (OF) or in integrated pest management (IPM). An untreated control without any foliar application was included in each field trial[a]

		Control treatments of *C. pomonella*		
Year/country/locality/IPM or OF	Apple cultivar	Control treatment 1	Control treatment 2	Control treatment 3
2009/Algeria/Ain Djasser/IPM	'Anna'	Sucrose 0.01%	Thiacloprid	Thiacloprid + sucrose 0.01%
2010/Algeria/Yabous/IPM	'Golden Delicious'	Sucrose 0.01%	Thiacloprid	Thiacloprid + sucrose 0.01%
2011/Algeria/Ain Djasser/IPM	'Golden Delicious'	Sucrose 0.01%	Thiacloprid	Thiacloprid + sucrose 0.01%
2013/France/Paluds de Noves/IPM	'Granny Smith'	Sucrose 0.01%	Thiacloprid	Thiacloprid + sucrose 0.01%
2013/Algeria/Tilatou/IPM	'Anna'	Sucrose 0.01%	Thiacloprid	Madex®
2013/France/Paluds de Noves/OF	'Gala'	Sucrose 0.01%	Carpovirusine™ 2000 + Carpovirusine® Evo2	Carpovirusine™ 2000 + Carpovirusine® Evo2 + sucrose 0.01%
2014/Algeria/Ain Touta/OF	'Starkrimson'	Sucrose 0.01%	Madex®	Madex® + sucrose 0.01%
2014/Algeria/Ain Touta/OF	'Royal Gala'	Sucrose 0.01%	Madex®	Madex® + sucrose 0.01%
2014/Algeria/Ain Djasser/OF	'Anna'	Sucrose 0.01%	Madex®	Madex® + sucrose 0.01%

[a] Common names of CpVG: Isolat GV-0006 = product Madex®; Isolat CpGV-M = product Carpovirusine™ 2000; Isolat NPP-R5 = product Carpovirusine® Evo2.

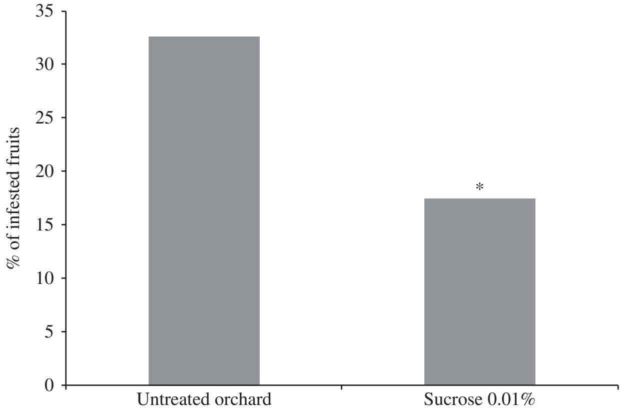

Figure 4. Comparison of the means of the percentage of infested fruits in apple orchards in Algeria and in France ($n = 8$). Control treatments of *C. pomonella* were as follows: foliar application of sucrose 0.01% according to Section 2.1.1 and the untreated control with no product applied. Bars represent standards errors. The asterisk (*) indicates differences according to Wilcoxon–Mann–Whitney, $P = 0.015$.

treatment. The CpGV reduced significantly the codling moth fruit damage, contrary to the sucrose application. Furthermore, the sucrose + CpGV treatments did not reduce fruit damage significantly compared with CpGV alone. The means of Abbott's efficacy (Table 2) for sucrose, CpGV and CpGV + sucrose were respectively 41.0 ± 10.0%, 51.8 ± 10.2% and 57.0 ± 8.0% and were significantly different for sucrose and CpVG + sucrose.

3.3 Effects of sucrose 0.01% on codling moth fruit damage in integrated fruit production

In integrated fruit production (or integrated pest management), the chemical insecticide thiacloprid and the sucrose treatment alone showed higher efficacies than the untreated modality (Table 1) in all field trials. The sucrose treatment was as efficient as or superior to the thiacloprid treatment in damage reduction. Indeed, the results in similar trials ($n = 4$) showed that the sucrose treatment (14.6 ± 4.2%) was equivalent in fruit damage reduction to the thiacloprid treatment (15.1 ± 5.4%) (Fig. 3) for two field trials in Algeria on the 'Anna' cultivar. Both treatments reduced the damage by more than half compared with the untreated control (37.4 ± 3.6%). In field trials in Algeria on the 'Golden Delicious' cultivar, thiacloprid was more efficient than sucrose. Furthermore, the sucrose treatment added to thiacloprid treatment enhanced the reduction in fruit damage compared with thiacloprid alone. The means of Abbott's efficacy (Table 2) for the treatments with sucrose, with thiacloprid and with thiacloprid + sucrose were respectively 41.0 ± 10.0%, 59.5 ± 12.8% and 77.9 ± 8.0% and were significantly different. The observed effect on efficiency was synergistic and more like a potentiation because the sucrose improved the efficacy of thiacloprid by 18.4%.

3.4 Practical efficacies of the addition of sucrose to thiacloprid or to the granulovirus treatments versus pesticides alone

Among the five treatments studied, the one that was the most improved by sucrose in apple orchards under agricultural production, organic farming and integrated fruit production (Table 2) was the thiacloprid (77.9 ± 10.6%). No significant differences were observed between CpGV, CpGV + sucrose and thiacloprid alone. The CpVG has comparable efficacy to chemical insecticide. The efficacy of sucrose 0.01% was significantly different from that of thiacloprid + sucrose and was slightly less than those of CpVG, CpVG + sucrose and thiacloprid alone.

4 DISCUSSION AND CONCLUSION

One objective was to induce immunity or resistance to the pest in agriculture as a complement and/or alternative to phytosanitary products. Hitherto, the use of sugars has concerned large molecules as polysaccharides added to formulations to facilitate their penetration through the cuticle. Very few trials have been reported to be consistent. Here, we show that small molecules such as sucrose can induce partial resistance to *C. pomonella* by foliar applications of aqueous solutions at the very low dose of 0.01%. Abbott's efficacy obtained for sucrose 0.01% alone was 41.0 ± 10.0% versus 36.1 ± 10.1% with sucrose 0.001% ($0.01\,\mathrm{g\,L^{-1}}$),[14] and no varietal effect was observed between the 'Anna' and 'Golden Delicious' cultivars. Increasing doses from 0.1 to 10 g per 100 L did not change the effects.[14] Higher doses could have a direct influence on the epiphytic flora or the epiphytic phase of some pathogens before contamination. Sucrose + thiacloprid was the most effective treatment; sucrose enhanced the efficacy of thiacloprid treatment by 18%. The effect of this combination is not additive but rather potentiating, because the sucrose increased the efficiency of the classic treatment. We can hypothesise that the insecticide or its formulation elicits signalling pathways, which are partly the same as those elicited by the sucrose alone. The commercial formulations of many phytosanitary products are introduced in order to improve the adhesion to the surface and the penetration through the cuticle. This could possibly lead to a risk of modification of the cuticular permeability and therefore of the composition of the blend and its effects on pest behaviour. On the other hand, we know that in the formulation of granulovirus there are simple sugars. They may elicit sucrose and/or fructose signalling pathways. In this case, it is likely that the addition of sucrose cannot enhance the induction of resistance already elicited by sugars in the formulation. We have already shown that the application of Madex® induced modifications of the blend of metabolites, with decreased egg laying, *C. pomonella* oviposition and larval performance.[56] Changes to this blend by the application of sucrose or fructose alone also reduce the number of egg-laying females and the intensity of egg laying for females that lay eggs.

The strategy of foliar applications of sucrose solution is rather simple and consists of application in the morning before 7 a.m. (solar time), just after its preparation, every 20 days. Birch *et al.*[57] observed regulation of defence signalling pathways (Jaz-1 marker) 4–6 h after foliar applications on tomatoes of D-fructose, D-glucose and sucrose. The delay in observing any effects on insect behaviour was longer, between 15 and 20 days, on *O. nubilalis*.[58] The duration of the resistance induced could last 20 days and had to be repeated to be maintained (unpublished on maize). The advantage of a rapid systemic signal induction and its impact for a relatively long period (20 days) facilitates the positioning of the treatment throughout the phytosanitary course and is less dependent on rainy days for applications. The partial effect of the resistance obtained, which may be equivalent to that of some products currently used against the codling moth in orchards, nevertheless needs to be improved. This could be done either by optimising the activity of sugars to their performance as a function of the plant growth stage when applied. The susceptibility of the cultivar to codling moth or by addition to other products. Other compounds from the formulation, such as sugars, are introduced into commercial products as phagostimulants for more effective ingestion by larvae. Many products can elicit plant reactions[59] and/or alter cuticular permeability.[60,61] Signalling pathways elicited by products may be common with those elicited by sugars; the worst case would be elicitation of antagonist signalling pathways to those elicited by the sugars.

The damage reductions achieved across several years, cultivars and countries with different climates and insect generation numbers confirm the robustness of our assumptions. Surveillance of *C. pomonella* populations will be important because it could in theory adapt its oviposition on apple trees treated with sucrose over several years. Moreover, direct or indirect effects on auxiliary and parasitic organisms cannot be completely excluded.

Recent research has led to the concept of 'sweet immunity' and 'damage self-recognition', in which sugar signalling pathways are involved. Small sugar sucrose transport and its perception are still being investigated. In our experiments, the quantities of sucrose applied on the leaves in the morning (best diffusion through the cuticle while the apoplast concentrations are low) are those usually found on apple leaves in the evening. Several days later this resulted in an induction of partial antixenosis at the *C. pomonella* egg laying step by modifications of the composition of sugars in blend. Several hypotheses can be advanced. A single sugar applied on leaves without any injury can induce a plant response and could be the origin of a stress or a self-damage signal. The output and input of the sugars through the cuticle follow the photosynthesis rhythm. Once sprayed, the sugars can penetrate the leaf by transcuticular hydrophilic pores[12] and induce some changes in apoplastic/cell sugar content and source-to-sink transition, increased cell-wall invertase activity and also changes in the sucrose/hexose ratio in plants.[17] At this point, plant immunity could be stimulated. Bolouri Moghaddam and Van den Ende[22] showed the importance of circadian rhythms in the regulation of sucrose-mediated signalling associated with immunity and abiotic stress response. Stress may be localised at the cuticle and/or stomata.[62,63] The majority of guard cells have chloroplasts, which would therefore provide an ideal and convenient location for sensory or regulatory mechanisms. Guard cell aploplastic sucrose can also exert an osmotic effect, which can lead to stomatal closure, acting as a possible signal between the mesophyll assimilation rate and transpiration.[64] It was postulated that sucrose concentrations near the guard cell regulate gene expression, as has been shown in many other tissues.[65] The leaf as the support at the origin of the stress is emphasised by experiments consisting in watering the soil with a fructose analogue, 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP), which induced root resistance in tomato against nematodes, but lower than by foliar applications.[66] This remains still to be studied. The resistances could be expressed at various levels and at different times against females and larvae.

The modification of egg laying of *C. pomonella* after sucrose applications correlates with unbalanced sugar and sugar alcohols at the leaf surface. Leaf surface blend signals indicate to females that the plant may be unsuitable for their offspring. Sorbitol is already known to play a role in biotic and abiotic plant responses, e.g. reactions to pathogen infections and tolerance to environmental stress.[67] Its high levels are a deterrent for *C. pomonella*, indicating a plant under stress. The situation here is even more complex, as the induced resistance consists in females avoiding a defence (priming) that should be triggered only by its progeny. The target reached and the time when this is achieved are two criteria that must be kept in mind in order to understand the mechanisms of plant immunity. The extremely complex networks in which sugars are involved suggest that resistance induction may be expressed in agronomic conditions under a multitude of stresses.

Host-specific non-pathogen-associated epiphytic microorganisms can induce the leaking of metabolites from plants[68] and/or produce them.[69] Their possible contribution to chemical signals given by the leaf surface is an issue that should not be ignored.[70,71] Chemical changes in the water-soluble metabolome in tissues and at plant surfaces owing to the application of the sugars are not entirely similar (unpublished), and we cannot exclude the effects of epiphytic microorganisms in the biochemical composition of leaf surfaces after sugar applications.

No related evidence between our results and the 'sweet immunity' concept has been provided in this paper. However, it should stimulate research on this concept for the development of biocontrol strategies. Further studies on, for example, invertase activity and/or hormonal (e.g. jasmonic acid) responses, as well as on PR levels, is needed to understand the different mechanisms involved in the plant. It will also be interesting to combine sugars with some reduced doses of chemicals. The experimental designs could be improved with a water application, which represents the best control treatment in our conditions. Indeed, as such a control treatment (i.e. foliar spray of water) could be perceived by the plant as an abiotic stress and could induce some defence reactions in the plant, it is not easy to discern a difference between the plant reactions induced in response to sucrose and those induced in response to foliar spray. In other experiments and in preliminary field experiments against *C. pomonella*,[12] D-fructose (0.01%) gave better results than sucrose. These field trials are going to continue, and many tests against pathogens and herbivory are programmed in the French project 'SWEET' (2016–2018) financed by the French Ministry of Agriculture.

Moreover, the EU approved sucrose in August 2014 (No. 916/2014) as a basic substance for plant protection purposes (for apple trees against *C. pomonella* and for maize against *Ostrinia nubilalis* at the vegetative stage). An application has been received from the Institute Technique de l'Agriculture Biologique in France for approval.

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