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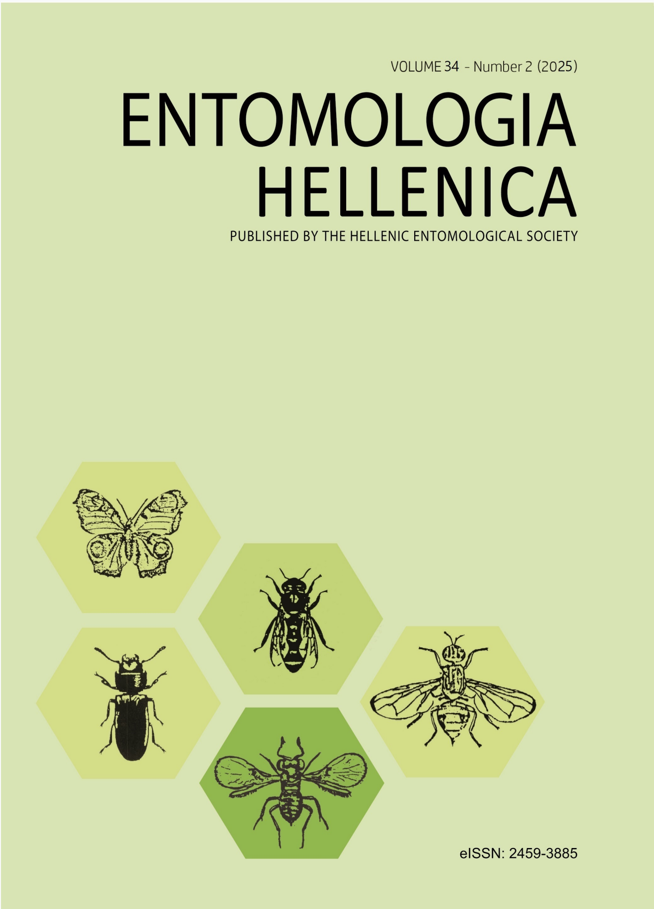

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Relationship between secondary nutrients of apple leaves and control of the Codling Moth (*Cydia pomonella* L., Lepidoptera: Tortricidae) by low doses of sugars

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ABSTRACT

The codling moth (*Cydia pomonella* L., 1758) regularly causes considerable damage in orchards, leading to extensive use of chemical insecticides. Many studies have focused on the relationship between nutrients and plant pests and diseases, and several sound reviews link the two issues. The aim of this study is to investigate the effect of sugar spraying at infra-doses on the nutrients present in apple leaves, in comparison with a control and a reference insecticide (deltamethrin), to understand the mechanisms leading to apple tree resistance to the codling moth. The results obtained highlighted that glucose, fructose and insecticide treatments significantly increased the concentrations of calcium, magnesium, boron, chlorides and iron compared to the untreated control. Additionally, the glucose treatment increased the concentration of manganese whereas it decreased the concentration of copper. In conclusion, infra-dose sugar spraying modified the nutritional status of the leaves, and this modification probably influences plant resistance to the codling moth.

KEY WORDS: *Cydia pomonella control, deltamethrin, fructose, glucose*.

Introduction

A host plant refers to a plant species that, under natural conditions, allows an insect to develop from egg to adult (accepted by both the adult female for laying eggs and the larva for feeding). The reproductive cycle of the insect must, therefore, coincide with the development of the host plant, and the insect must have effective mechanisms to locate and identify it. For the lepidopteran species, the adult female chooses the feeding source for its offspring (Renwick & Chew, 1994). The selection of the host plant by an insect (for feeding, laying eggs, shelter, or refuge) is based on the physical (colour, odour, texture, etc.) and chemical characteristics (nutritional status, presence or absence of toxic metabolites, etc.) of the host plant (Mookiah et al., 2021). With specifically adapted sensory equipment, the insect detects chemical and physical signals

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produced by the plant through olfaction and vision from a distance, and through taste and mechanical reception upon contact with the plant tissues. Integration of these different signals at the central nervous system (CNS) forms the sensory image, which will be compared with the image stored and genetically fixed in the CNS of the insect, that can be modified through experience (Schoonhoven et al., 1998). Through repeated contacts of legs, antennae, ovipositor, and mouthparts with the plant surface, the insect attempts to perceive physical and chemical signals indicating the acceptability of the site (Maher, 2002).

Primary metabolites play a role as recognition signals for females. Lombarkia and Derridj (2002) revealed that the primary metabolites present on the leaf surface act as kairomones. Certain primary metabolites such as fructose, sorbitol, and myo-inositol influence the acceptance and egg-laying stimulation of the codling moth *Cydia pomonella* (Lombarkia & Derridj, 2002). Derridj et al. (1999) highlighted the effect of sugars (glucose, fructose, sucrose) and sugar alcohols (sorbitol, quebrachitol, and myo-inositol) on the egg-laying behaviour of females and the neonate larvae of *C. pomonella*. Primary metabolites also stimulate food intake. Indeed, Schoonhoven et al. (1998) focused on the carbohydrates and amino acids produced by all plants as a result of their photosynthetic activities. Most herbivorous insects primarily use carbohydrates as feeding stimulants. For most species studied, sucrose, glucose and fructose are the most potent stimulants, present at 2 to 10% of dry weight. Besides, secondary metabolites derive from the secondary metabolism and are the best representatives of a plant species, as they can be characteristic for different plant genera or families either by their quantitative dominance (such as in the case of many phenolic compounds and flavonoids) or by their uniqueness. In this case, secondary metabolites are specific to a particular taxon and act as feeding or egg-laying stimulants for monophagous or oligophagous species (Schoonhoven et al., 1998).

Lombarkia and Derridj (2008) demonstrated the important role of sugars in the preferred egg-laying site on apple trees for *C. pomonella* and its egg-laying stimulation was linked to a mixture of sucrose, D-fructose, glucose, sorbitol, quebrachitol, and myo-inositol present on the surface of the apple leaves. The exogenous foliar application of sucrose and D-fructose can induce resistance by antixenosis to insect egg-laying. Foliar applications of sugars every 20 days on apple trees to reduce *C. pomonella* damage have been practiced in commercial orchards in several countries (southern France, Italy, Greece and Algeria) over several years, showing an effectiveness of 40 to 59% (Derridj et al., 2011).

Thus, the aim of the present study is to assess the influence of infra doses of sugars on the leaf mineral composition.

Materials and Methods

Experimental design. The experiment was carried out in 2021 at a ‘Royal Gala’ apple orchard (35°21′21,6″ N, 006°01′ 16,5″ E) in the province of Batna (eastern Algeria). The trees were 11 years old, grafted on MM 109, planted at a 4 × 4 m grid, managed under the common practices of the area. During our study, the experimental plot did not receive any plant protection treatments except for micronized sulphur 80%, which is authorized in biological control programs and was applied at a concentration of 750 g in 100L water, every 20 days, for a total of seven times.

The experiment was based on a randomized latin square experimental design with 4 repetitions. All treatments were distributed within each block, including three trees. The treatments were applied using an electrical pressure sprayer (Zeralda Electric Yellow A012) of a 16 L capacity. The tested modalities were fructose and glucose against the commercial insecticide Decis (a.i. deltamethrin), in addition to an unsprayed control. The application of sugars (glucose 100 ppm and fructose 100 ppm) was carried out immediately after the solution preparation, early in the morning, when the apoplast (intercellular space) and the leaf surface are low in sugars, and applications were repeated every 20 days, from the end of flowering to harvest, according to Derridj et al. (2012). The insecticide was applied at the recommended dose of 8 ml/16 L of water every 20 days

Leaf sample collection after treatments for biochemical analyses. Sample collection was carried out on both control and treated trees. Sample collection period corresponded to the peak egg-laying period of the fourth flight of the insect. We considered that one sample represents one repetition. A "leaf" sample consisted of 4 leaves surrounding the fruits on one branch (Lombarkia, 2002). Samples were collected with non-powdered latex glove protected hands to avoid contamination of the samples by substances (sugars) present on the hands, and were subsequently placed in polystyrene containers, after being separated from each other with Wattman filter paper. To prevent any contamination by sap, the cut parts were immediately wrapped in aluminium foil.

Analysis of anions and cations. The quantification of cations (calcium, magnesium, manganese, boron, iron, copper) and anion (chloride) was performed according to the methods mentioned in Table 1.

TABLE 1. Methods used to measure minerals

Mineral Element (unit of measurement)	Method
Calcium Ca^{+2} (mg/kg)	Flame Photometer
Magnesium Mg^{+2} (mg/kg)	Titrimetry NFT 90-005
Iron Fe (mg/kg)	Triazine Method
Copper Cu^{+2} (mg/kg)	Cuprizone Method
Manganese Mn (mg/kg)	Method P.A.NDIN 38406-E2
Boron B^{+3} (mg/kg)	Carmin Method
Chlorides Cl^{-} (mg/kg)	Silver Nitrate Method NFT 90-014

Statistical analysis. The statistical procedure for the obtained data was performed with SPSS software. The means of the variables calcium, magnesium, manganese and copper were compared by Kruskal-Wallis test, while iron, boron and chlorides were compared by ANOVA test, followed by post hoc analysis using Fisher's and Tukey's tests. A P-value of 0.05 was used to establish statistical differences in all tests.

Results

Calcium concentration. Foliar sprays of glucose, fructose and the insecticide increased significantly the calcium concentration compared to the control. The analysis of variance (Kruskal-Wallis test) ($P < 0.05$) identified two groups: control (7767,76 mg/kg, a group), followed by the spraying of glucose, fructose and insecticide (21218,5 mg/kg, 21175,23 mg/kg and 12565,46 mg/kg, respectively b group) (Table 2).

Magnesium concentration. The spraying of glucose and fructose induced a significant increase in magnesium concentration compared to the untreated control, and their percentage was similar to that of the insecticide. The analysis of variance (Kruskal-Wallis test) ($P < 0.05$) identified two groups: control (3483,4 mg/kg, a group), followed by the spraying of glucose, fructose, and insecticide (5287,6 mg/kg, 5173,63 mg/kg and 4570,6 mg/kg, respectively (b group) (Table 2).

TABLE 2. Mineral concentrations (n = 12) on different modalities (control, fructose, glucose, insecticide). Different letters in the same line indicate a significantly different calcium amount ($P < 0.05$).

Treatments	Control	Glucose	Fructose	Insecticide
Calcium	7767.76±311.41 a	21218.5±112.65 b	21175.23±935.63 b	12565.46±1305.29 b
Magnesium	3483.4±441.88 a	5287.6±1259.78 b	5173.63±1574.19 b	4570.6±474.32 b
Manganese	1633.33±72.64 a	2650±76.37 b	1800±76.37 a	1916.67±44.09 a
Boron	528±39.71 a	978±7.57 b	881.67±39.19 b	438.67±11.62 a
Chloride	10393.33±483.64 a	17040±1477.98 b	29826.67±819.86 c	15146.67±1252.32 b
Iron	44.13±0.35 a	57.73±2.88 b	58.26±2.14 b	56.4±1.85 b
Copper	121.67±1.66 a	86.67±12.01 b	121.67±6.01 a	81.67±8.81 b

Significance at $P < 0.05$

Manganese concentration. Foliar sprays of glucose induced a significant increase in manganese concentration compared to the untreated control. On the other hand, fructose generated a concentration similar to that of the insecticide. The analysis of variance identified two groups: control, fructose and insecticide (1633,33 mg/kg, 1800 mg/ and 1916,67 mg/kg, respectively a group), followed by the spraying of glucose, (2650 mg/kg, b group) (Table 2).

Boron concentration. The spraying of glucose and fructose induced a significant increase in the boron concentration (978 mg/kg and 881,67 mg/kg, respectively) compared to the untreated control and the insecticide (528 mg/kg and 438,67 mg/kg, respectively (Table 2).

Chloride concentration. Foliar sprays of glucose, fructose and the insecticide induced a significant increase in chloride concentration compared to the untreated control. The analysis of variance (ANOVA) followed by the Tukey test ($p< 0.05$) identified three groups: control (10393,33 mg/kg, a group), glucose and insecticide (17040 mg/kg et 15146,67 mg/kg, respectively b group), followed by the spraying of fructose (29826, 67 mg/kg, c group) (Table 2).

Iron concentration. The analysis of variance revealed a significant difference between the tested modalities classified into two groups: control (44,13 mg/kg, a group), followed by the spraying of glucose, fructose, and insecticide (57,73 mg/kg, 58,26 mg/kg and 56,4 mg/kg, respectively, b group) (Table 2).

Copper concentration. The spraying of glucose and the insecticide induced a significant decrease in the copper concentration compared to the untreated control. The analysis of variance (Kruskal-Wallis test) divided the treatments into two groups: control and fructose (121,67 mg/kg and 121,67 mg/kg, respectively a group), followed by the spraying of glucose and insecticide (86,67 mg/kg and 81,67 mg/kg, respectively b group) (Table 2).

Discussion

According to Derridj and Wu (1996), the European corn borer (*Ostrinia nubilalis*) lays its eggs on the levels and leaf faces of corn that are less rich in cations (calcium, sodium, potassium). Additionally, sequential effects have been observed between sugars and cations, showing that depending on the concentration, either substance can be dominant in the observed behavioural response. The same authors demonstrated that a high concentration of cations has a dominant effect over that of sugars, and a synergistic effect between cations and sugars has been observed. Furthermore, Tiffrent and Lombarkia (2022) showed that glucose and fructose treatments significantly reduced the number of eggs and led to similar to the reference chemical treatment results, during the first and third generation flights for the 'Golden Delicious' variety and during the fourth flight for 'Royal Gala'. These results encouraged us to investigate the effect of infra-dose sugar spraying on the cations present in apple tree leaves, in order to understand the mechanisms leading to resistance against *C. pomonella*.

Mineral nutrients play a critical role in the growth and development of both plants as well as microorganisms. They are key factors in the interactions between plants and diseases, influencing how each nutrient affects a plant's response to disease (Spann & Schumann, 2010). In addition to carbon, hydrogen, and oxygen, which plants derive from carbon dioxide and water, 14 essential nutrients are identified. These include primary macronutrients (nitrogen, phosphorus, and potassium), secondary macronutrients (calcium, magnesium and sulfur), and micronutrients (iron, manganese, zinc, copper, boron, molybdenum, chlorine, and nickel), all of which are crucial for plant growth (Bala et al., 2018). In our work, the spraying of sugars at low doses modified the nutritional status of the leaves. This modification likely influences plant resistance to the codling moth. This hypothesis is supported by Spann and Schumann (2010), who indicate that mineral nutrition influences growth and yield by affecting plant resistance or susceptibility to pathogens and pests. Although resistance is genetically controlled, it is greatly influenced by environmental factors, among which mineral nutrition can increase plant resistance by responding to pest attacks through two mechanisms: the formation of mechanical barriers mainly by developing thicker cell walls and the synthesis of natural defenses, such as antioxidants and flavonoids, which offer protection against pathogens. According to Maffei et al. (2007), successful plant defense depends on the plants' ability to quickly recognize an enemy. The initial defense responses require signaling cascades initiated by the enemy. Their activation ensures a quantitative, localized and coordinated with other activities response of the host cells. Ionic imbalances induced by damage and modulations of channel activities are the first events occurring in the plasma membrane, involving variations in Ca^{2+} concentration. Downstream interactions with kinase networks and phytohormones mediate the signal, leading to a concerted activation of genes.

In the present study, glucose, fructose, and insecticide spraying significantly increased calcium, magnesium, boron, chlorides and iron concentration. Calcium (Ca) ions play a role in signaling pathways, as indicated by Pszczolkowski (2017), who demonstrated the inhibitory effect of denatonium on the feeding of neonate codling moth larvae. This inhibition relies on signaling pathways involving phospholipase C, phosphodiesterase, and calcium ion influx into the cells. Furthermore, Derridj and Wu (1996) highlighted the role of primary metabolites and cations present on the leaf surface in selecting the oviposition site and the sensory perception of the corn borer. For secondary macronutrients, calcium is used for cell division and formation, involved in nitrogen metabolism, the translocation of photosynthesis from leaves to fruiting organs, reduces respiration, and increases fruit set. In addition, Mazumder et al. (2021) revealed that among four tomato varieties examined, one variety (MT-3) responded well to calcium chloride weekly (from fruit formation to first harvest) applications at a dose of 2% ($CaCl_2$), by improving growth, yield and fruit quality, as well as reducing the severity and impact of diseases and insect infestation. The stimulating properties of calcium chloride on the consumption of apple leaves by newly hatched codling moth larvae disappear in the presence of increasing concentrations of the calcium chelator EDTA, indicating that it is the calcium cations, not the chloride anions, that actually increase leaf consumption (Pszczolkowski, 2003). However, high concentrations of calcium generally caused the rejection of food intake by the larvae of Samia cecropia (Frings, 1945, 1948 in Pszczolkowski, 2003).

Moreover, magnesium (Mg) is a key element in chlorophyll production; it improves phosphorus utilization, and it is an activator and component of many plant enzymes, enhances iron utilization and influences earliness and uniformity of maturity (Shukla et al., 2014).

For microelements, boron (B) is indispensable for the germination of pollen grains, essential for grains and cell walls,

Chlorine, in the form of a charged ion (Cl^-), is essential for oxygen evolution in photosynthesis, has a synergistic effect with potassium, and combines to form KCl, which is effective in reducing the severity of plant diseases and water stress. It is known as a nitrification inhibitor and regulates osmosis (Shukla et al., 2014).

Furthermore, iron (Fe) plays an important role for plants in various physiological functions and biochemical pathways. It serves as a component of many vital enzymes, is involved in chlorophyll synthesis and has an essential role in metabolic processes such as DNA synthesis, respiration, and photosynthesis (Rout & Sahoo, 2015).

Manganese (Mn) is an essential micronutrient with many functional roles in plant metabolism. It acts as an activator and cofactor of hundreds of metalloenzymes in plants. Due to its ability to easily change oxidation states in biological systems, it plays an important role in a wide range of enzyme-catalyzed reactions (Schmidt & Husted, 2019).

and promotes maturity. It is necessary for sugar translocation and affects the metabolism of nitrogen and carbohydrates. Furthermore, a study conducted by Ruuhola et al. (2011) on the resistance of birch seedlings to the larvae of the autumnal moth (*Epirrita autumnata*) showed that boron fertilization improved resistance, which was mirrored by a reduction in the pupal weight.

Treatments of apple trees with glucose and fructose increased the concentration of most elements, namely calcium, magnesium, chloride, boron, and iron. Additionally, glucose treatment increased the concentration of manganese while it decreased the concentration of copper. In this context, Ribeiro et al. (2017) showed that some plant species evolved in high-altitude grasslands developed adaptations for these extreme conditions, such as metal accumulation and found that concentrations of manganese and iron in the leaves significantly affect insect herbivory activity.

Conclusion

In conclusion, the application of sugars at ultra-low doses altered the nutritional status of apple leaves, inducing variations in the quantities and proportions of secondary macronutrients and micronutrients. This modification seems to be contributing to the plant's resistance against the codling moth and consequently reducing the need for chemical insecticide applications.

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