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3, 4-Dihydroxyphenylethanol, A Potential Repelling Compound Implicated in the Interaction of Olive Tree-Psyllid

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Abstract: *Euphyllura olivina* Costa (Psyllid) is a pest insect causing considerable wastes of crops by provoking the losses of young buds, flower buds and fruits in *Olea europaea* L. It seems that cultivars influence the oviposition performance of Psyllid. A certain degree of resistance to olive Psyllid in young and flower buds was associated with higher level of phenolic compounds and particularly the level of 3, 4-dihydroxyphenylethanol (3, 4-DHPE). In this study, analysis was performed on the evolution of the level of 3, 4-DHPE in young and flower buds infested or not by Psyllid and the effects of this compound on the oviposition performance and insect mortality on young buds as well as on their phenolic compounds. The infestation by Psyllid decreased the level of 3, 4-DHPE; this reduction is more pronounced in flower buds in comparison with the young buds (83.9 and 20%, respectively, after 30 days of treatment). The treatment with 3, 4-DHPE arrested the oviposition of Psyllid on young buds and induced a higher percentages of the insect mortality. No qualitative or quantitative changes of bud phenolics have been induced with 3, 4-DHPE treatment.

Key words: *Olea europaea* L., *Euphyllura olivina* Costa, phenolics, 3, 4-dihydroxyphenylethanol, plant-insect interaction

INTRODUCTION

Olive tree (*Olea europaea* L.) has a marked socio-economic impact in the mediterranean basin. The Olive culture is, however, limited by numerous factors and insect pests. Among the feeding insects, Psyllid: *Euphyllura olivina* Costa (Hemiptera *Psyllidae*) causes considerable wastes of crops by provoking the losses of young buds, flower buds and fruits. Without any chemical treatment, severe losses occurred in olive production annually and may reach 65% by this disease that we can here name for the first time 'Psyllose'. These losses are correlated to the degree of multiplication of Psyllid and abundance of larvae particularly at the end of their biological cycle development needing more predation and food. In addition, the insect mucilaginous production that covers the injured plant material affects photosynthesis and constitutes attracting site of other parasites, such as fungi.

The insect life stages are extremely related to the phenological phases of the host plant[1] and the number of annual generation varied according to the season and region[2].

It seems that the cultivar influences the oviposition performance of Psyllid[3]. However, nothing has been known about the biochemical and molecular mechanisms implicated in this interaction olive tree-Psyllid. The previous work on this subject has shown that phenolics may have a certain role in this interaction[4,5]. Phenolics are natural products exhibiting diverse biological functions throughout the plants such as protecting them from pathogen and predator attack, providing a certain degree of the stabilization of cell wall cross-links and protecting lipids from peroxidation as they are natural antioxidants. These compounds constitute one among others playing a direct or indirect role against insects[6-9]. In olive tree, we have shown that a certain degree of resistance to Psyllid was associated to the higher levels of soluble phenolic compounds in young and flower buds[4,5]. Among several phenolics characterized, a particular attention is attributed to 3, 4-dihydroxyphenylethanol (3, 4-DHPE) as a compound implicated in the synthesis of oleuropein and verbascoside (two natural compounds largely represented in olive) and widely distributed in tissue. This compound is also present in the olive oil and fruits, with a high antioxidant capacity[10].

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Present aim was to better understand and to determine the link of feeding strategy of Psyllid to phenolics metabolism in olive tree. This paper shows the evolution of the level of 3, 4-DHPE in young and flower buds infested or not by Psyllid and the effects of treatment with this compound on the oviposition performance and insect mortality on young buds as well as on their phenolics.

MATERIALS AND METHODS

Plant material and mode of infestation: Among several cultivars studied (Santa Catharina, Gordale, Americana, Arbequine, Lucques and Frontoio) in this work, the more susceptible Santa Catharina cultivar to Psyllid is used for treatments. This choice may allow us to determine the protector effect of 3, 4-DHPE with regard to Psyllid.

Two tests have been performed: the first one was carried out using the young and flower buds and samples were infested artificially by Psyllid and collected 5, 15, 20 and 30 days after infestation. These were compared to the control samples collected at the same time, but without infestation. In the second test, three treatments were applied. In the first group, the young buds were sprayed with water and kept uninfested as control. In the second group, the young buds were infested by Psyllid and sprayed with water. The third group, was infested by Psyllid and 3, 4-DHPE was applied by spraying with in concentration 1 mM. After 15 and 30 days, the number of Psyllid eggs and adult mortality were determined. In the two tests the infested and uninfested materials were collected, washed and stored at -20°C for phenolics analysis.

Extraction and analysis of phenolics: Phenolic compounds were extracted and analyzed as described earlier[11,12]. Briefly, frozen tissues were extracted with 80% aqueous methanol at 4°C with continuous stirring. The extracts were purified and finally dissolved in methanol (HPLC grade) after evaporation in vacuo at 32-35°C. Phenolics analysis was performed using spectrophotometric, thin layer (TLC) and high performance liquid (HPLC) chromatographic methods.

TLC was conducted on microcristalline silice plates in the upper phase of chloroform/ethyl acetate/methanol: 8/1/2: v/v/v; first migration and ethyl acetate/acetic acid/water: 8/1/1: v/v/v; second migration. UV light (256-336 nm) was used with or without ammoniac vapor as well as other specific phenolics reagents such as Benedickt and diphenyl boric acid 2-aminoethyl ester (NEU) reagents.

Quantitative and qualitative analysis were carried out with a waters 600 E liquid chromatograph equipped with waters 990 photodiode array detector and a Licrosorb C-18 reversed phase column.

Phenolics were detected at three wave lengths 280, 320 and 350 nm and identified by their UV spectra, retention times and co-chromatography with commercial standards. The HPLC results were accompanied by phenolic spectrophotometric analysis using the Folin Ciocalteu reagent[13]. Each value represents a mean of three analysis; the standard error ranged from 2 to 4.3%.

RESULTS

When the more and less attractive cultivars to Psyllid were compared, it has been shown that phenolics levels were approximately 2 times higher than in the less attacked cultivars (Fig. 1). The same result has been revealed when the bud flowers (more attractive) were compared to the young vegetative buds (Fig. 2). During the flower period, invading herbivorous pests selectively attack the bud flowers and this predation results in severe losses in fruit production annually. These buds have a distinct phenolics profiles in comparison with those of the vegetative buds. These differences are attributed to several phenolics; 12 among them have been identified. Figure 3 revealed that 3, 4-DHPE is a compound positively related to the degree of 'resistance' of cultivars to Psyllid. This compound, widely represented in olive, is implicated in the synthesis of oleuropein and verbascoside and it has been found that it is highly accumulated in the less attractive cultivars to the Psyllid (Fig. 3). In addition, this compound shows a higher accumulation between February and April (Fig. 4) and a reduction particularly during the flowering and fruit formation periods. This lower level is accompanied by a higher multiplication of Psyllid.

Levels of 3, 4-DHPE in young vegetative and flower buds infested or not by Psyllid: During one month, the level of 3,4-DHPE increased in the control flower buds while it decreased in infested ones (Fig. 5). This level became approximately four times higher after 30 days of infestation in control. In infested young buds, the level of 3, 4-DHPE seems to be 2 times lowered after 15 and 30 days of infestation.

Table 1: Impact of 3, 4-DHPE on the mortality and oviposition performance of Psyllid

	Mortality		Oviposition performance	
Treatments	15 d	30 d	15 d	30 d
Water	26%	34%	20 eggs	92 eggs
3, 4-DHPE	44%	80%	0	0

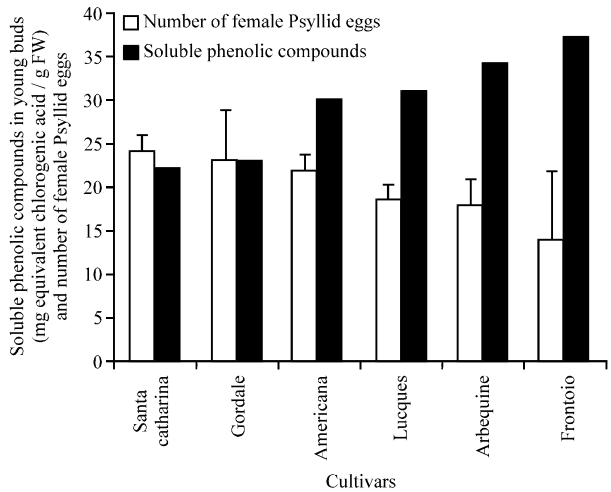

Fig. 1: Comparison of oviposition potential of Psyllid revealed by the number of eggs during 90 days of infestation and total phenolic compounds in young buds of 6 cultivars of olive tree. The standard errors for phenolics were 2-6%

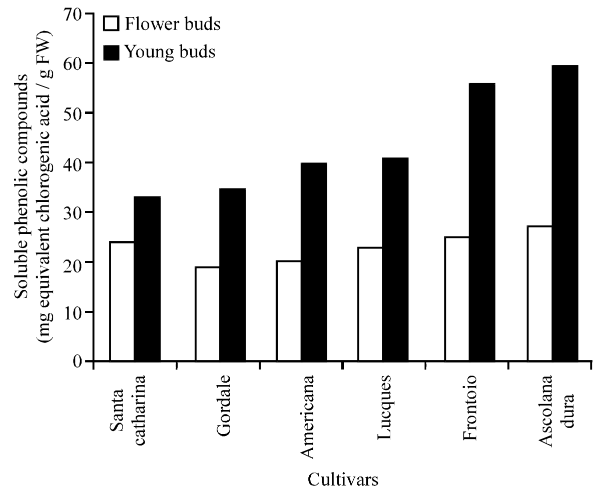

Fig. 2: Comparison of the levels of soluble phenolic compounds in young and flower buds of 6 cultivars of olive tree during the flower period. The standard errors for phenolics were 2-4.3%

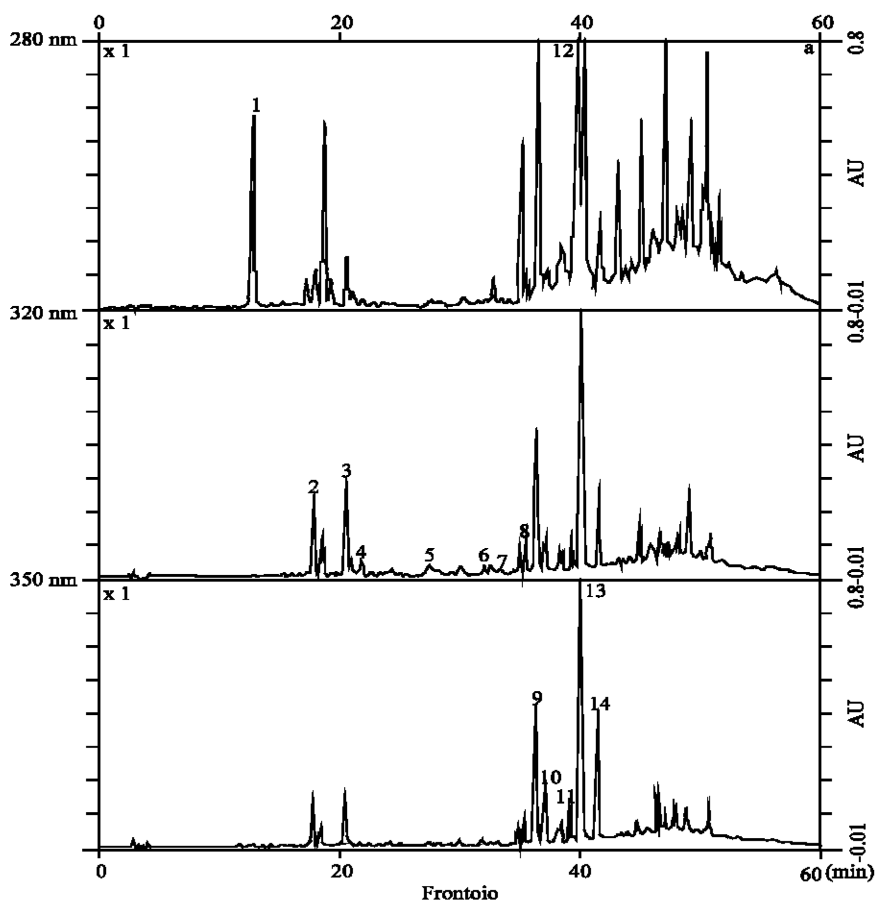

Fig. 3a: HPLC chromatograms of soluble phenolics in young buds of Frontoio 1: 3,4-dihydroxyphenylethanol; 2 and 7: caffeic derivatives; 3: chlorogenic acid; 4 and 5: p-coumaric derivatives; 6: hydroxycinnamic derivative; 8: verbascoside; 9: rutin; 10: luteolin-7-glucoside; 11: isoquercetrin, 12: oleuropein; 13: Isorhamnetin derivative; 14: flavonoid

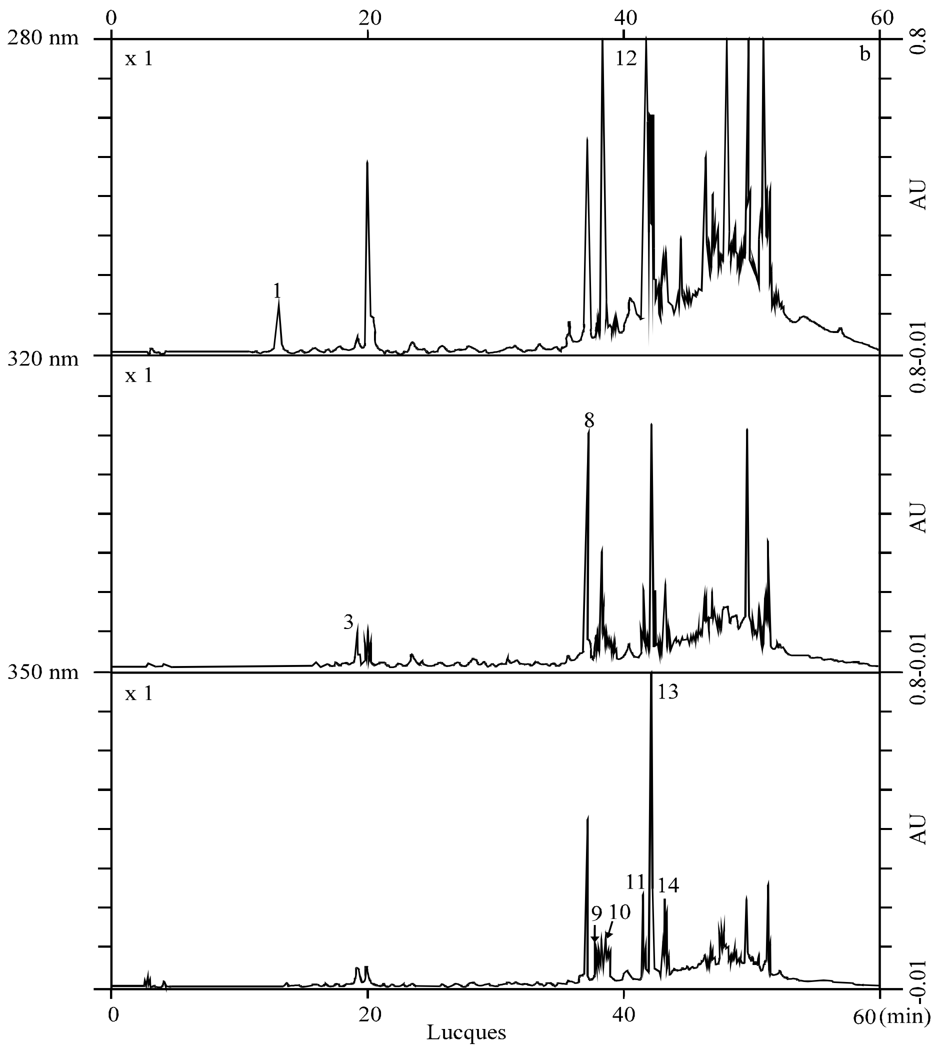

Fig. 3b: HPLC chromatograms of soluble phenolics in young buds of Lucques 1: 3,4-dihydroxyphenylethanol; 2 and 7: caffeic derivatives; 3: chlorogenic acid; 4 and 5: p-coumaric derivatives; 6: hydroxycinnamic derivative; 8: verbascoside; 9: rutin; 10: luteolin-7-glucoside; 11: isoquercetrin, 12: oleuropein; 13: Isorhamnetin derivative; 14: flavoinoid

Effect of 3, 4-DHPE on Psyllid and on endogenous phenolics in young buds: The experimental approach used to determine the possible protector effect of 3, 4-DHPE consists to treat with the sites attacked by Psyllid. The treatment with 3, 4-DHPE twice a month (1 mM) arrested the oviposition of Psyllid on young buds and increased the insect mortality (Table 1). These reached 44% after 15 days and 80% after 30 days of treatment. No qualitative or quantitative changes of bud phenolics have been induced with 3, 4-DHPE treatment. The same HPLC chromatograms were obtained with and without exogenous 3, 4-DHPE application. This shows that 3, 4-DHPE acts directly on Psyllid and not indirectly by changing endogenous phenolics.

DISCUSSION

The results show that the level of 3, 4-DHPE was 4 to 5-fold higher in the less than in the more attacked cultivars and could be related to the differences observed in the mortality and oviposition of Psyllid on cultivars studied. The cultivars of olive tree partly susceptible contained higher level of soluble phenolics in comparison with the more susceptible one. These results are in agreement with those obtained by Havlickova *et al.*[8] revealing that seedlings of susceptible Wheat cultivar Zdar contained lower level of free phenolic acids and were more infested by aphids than partly resistant cultivar Regina. A negative correlation exists between the degree

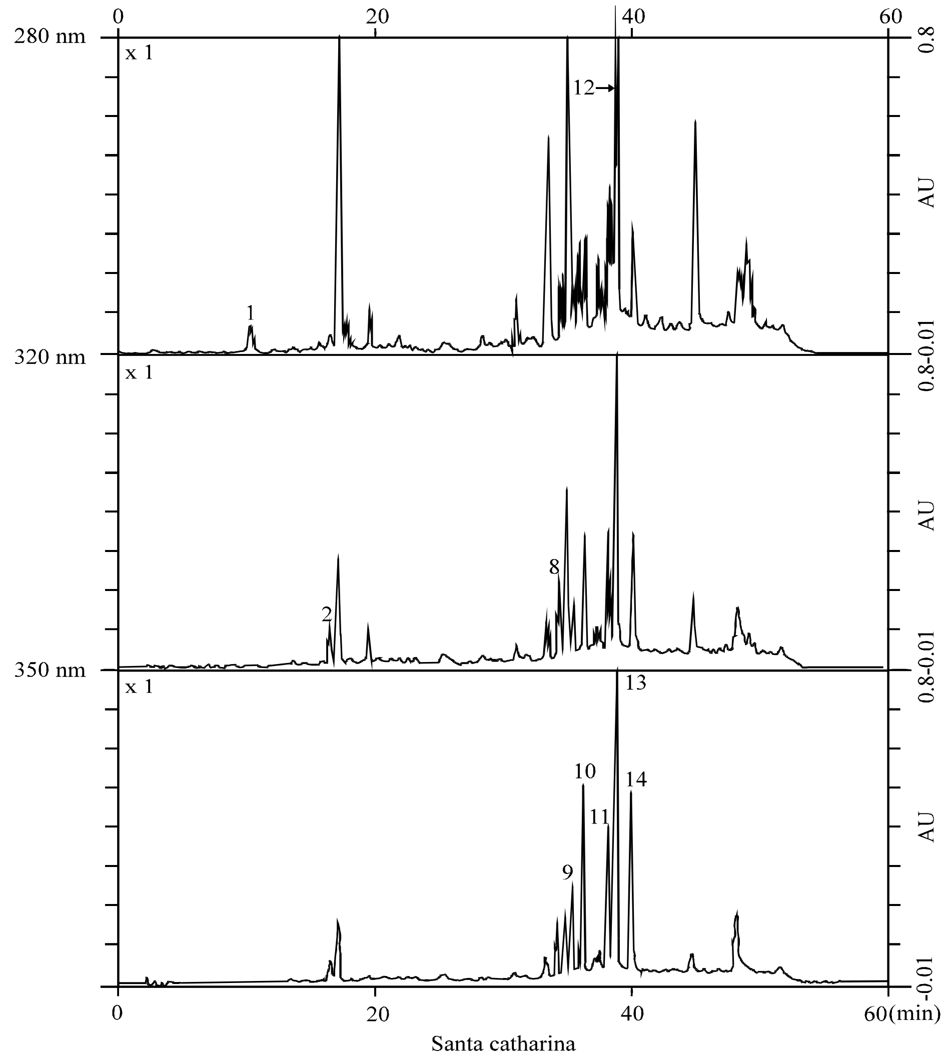

Fig. 3c: HPLC chromatograms of soluble phenolics in young buds of Santa Catharina 1: 3, 4-dihydroxyphenylethanol; 2 and 7: caffeic derivatives; 3: chlorogenic acid; 4 and 5: p-coumaric derivatives; 6: hydroxycinnamic derivative; 8: verbascoside; 9: rutin; 10: luteolin-7-glucoside; 11: isoquercetrin, 12: oleuropein; 13: Isorhamnetin derivative; 14: flavoinoid

of susceptibility of olive tree cultivars to Psyllid and the level of soluble phenolics. The same correlation has been observed in winter wheat cultivars with regard to the aphid attack[14]. In addition, flower buds, which are the preferential sites of attack during the flowering period, showed 2 to 3 times lower levels of phenolics than young buds in Olive tree. In comparison with control, flower buds decreased their content of 3, 4-DHPE. These are approximately 2, 3, 5 and 4 times decreased, respectively after 5, 15, 20 and 30 days of infestation. This situation may constitute a reaction of tissue to insect attack. It could be explained by oxidation of 3, 4-DHPE to o-quinone (3, 4-quinonephenylethanol) playing an antifeeding role. In Plumbago, Banerjee *et al.*[15] have shown that Plumbagin (a naphtoquinone) has a great insect growth disturbing activities. Similar results were described by Stevenson *et al.*[16] indicating that the weight of *Nilaparvata lugens* (planthoppers) decreased when they were exposed for a 25 h to rice stems with high levels of flavonoids. In addition, it was reported that the artificial diet of *Nilaparvata lugens*, containing apigenin-C-glycoside increased the mortality of nymphs[16] and quercetin-3-O-(2-ß-D-xylopyranosylrutinoside) deterred the oviposition of *Papillos xuthus*[9].

It has been suggested that feeding insects may utilize some strategies keeping to a minimum the activation of host defense genes. In the case of Olive-Psyllid interaction, it could be assumed that phenolic compounds

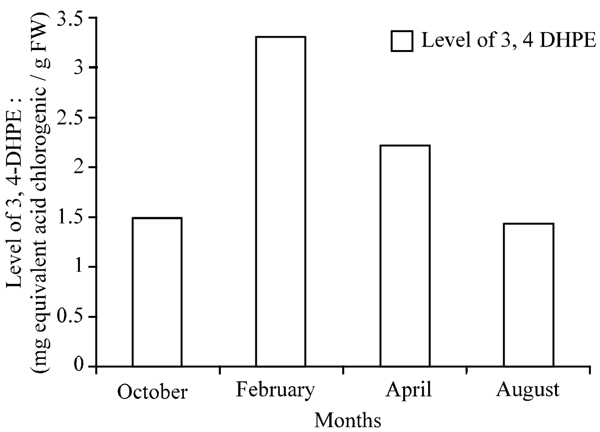

Fig. 4: Evolution of the levels of 3, 4-DHPE in young buds of Frontoio during four seasons

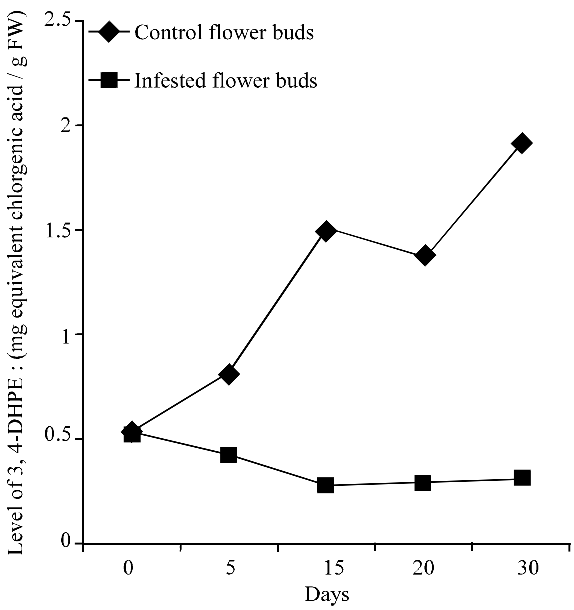

Fig. 5: Evolution of the 3, 4-DHPE levels in control and infested flower buds in olive tree

and particularly 3, 4-DHPE levels might influence the Psyllid oviposition. Some tests were conducted to evaluate the protecting effects of this compound. In general, treatments of young buds by spaying the product arrested the oviposition of insect and induced a higher level of its mortality. Similar results were described by Gariboldi *et al.*[17] showing that secoiridoides in *Olea europaea* and particularly 3, 4-DHPE produced by degradation of oleuropein exhibit an inhibiting effect with regard to oviposition of *Bactrocera oleae* (Rossi). In *Prunus serotina*, some bioactive compounds such as naringenin, methoxynaringenin and eriodictyol have antifeeding effects[18].

The 3, 4-DHPE constitutes a potential compound implicated in the interaction Olive-Psyllid. This compound might act directly as a potent inhibitor of insect proteinase or insect amylase or indirectly by activating the proteinase genes in the host-plant to prevent feeding insect. This may explain the lower oviposition performance of the insect as well as the higher percentage of its mortality. The higher phenolics levels in the less attractive cultivars to Psyllid might be also correlated with their physical barriers that enhance the degree of their resistance. The insecticidal activity of 3, 4-DHPE may constitutes a new approach for olive protection against Psyllid.

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