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# IMIDACLOPRID APPLIED THROUGH DRIP IRRIGATION AS A NEW PROMISING ALTERNATIVE TO CONTROL MEALYBUGS IN TUNISIAN VINEYARDS

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**Abstract:** Mealybugs are serious insect pests in Tunisian vineyards where they can cause major production losses. Thus, a management program of these insects is a priority for grape growers. A summer pesticide trial was conducted in a vineyard, located in the Cap-Bon Region of Tunisia. The trial was carried out to assess the use of imidacloprid, a systemic insecticide, against mealybugs on vine. Imidacloprid was applied through the drip irrigation system for each vine and was then compared to methidathion, a contact insecticide. Imidacloprid was found to be more effective than methidathion on all mealybug developmental stages. In addition to its outstanding, up to 100% efficiency, imidacloprid provided an interesting long-term control of mealybugs. No significant difference was found between the two imidacloprid rates (1 and 2 ml/vine). Methidathion generated an overall low to intermediate efficacy on mealybugs and was more effective on both first instar nymphs and adult females than on the other mealybug developmental stages. Thus, imidacloprid applied through a drip irrigation system is a new promising option to control mealybugs in vineyards. For this reason it can be employed in an integrated management program against these pests in the Tunisian grape-growing area.

**Key words:** Tunisia, vineyard, mealybugs, imidacloprid, drip irrigation system, Integrated Pest Management

## INTRODUCTION

Mealybugs (Hemiptera: Pseudococcidae) are considered among the most important plant sap-sucking insects within several agricultural areas of the world. This is especially true in vineyards where they are known as serious pests (Charles 1993; Ben-Dov 1994; Godinho and Franco 2001; De Borbòn *et al*. 2004; Walton *et al.* 2004; Miller *et al*. 2005; Daane *et al*. 2006; Buonocore *et al.* 2008; Zada *et al.* 2008).

In Tunisia, two mealybug species were recorded on vine. They are the vine mealybug *Planococcus ficus* Signoret and the citrus mealybug *Planococcus citri* Risso (Mansour 2008; Mahfoudhi and Dhouibi 2009; Mansour *et al.* 2009).

These species produce honeydew that drops on the grape bunches and promotes the development of the black sooty mold fungi which decrease grapevine quality. In addition, mealybugs are vectors of viral diseases of grapevines (Rosciglione *et al*. 1983; Cabaleiro and Segura 1997; Tsai *et al.* 2008; Mahfoudhi *et al.* 2009).

To limit crop losses due to mealybug activity in vineyards, different pest control methods are used in several grape-growing regions of the world.

Among these methods, insecticide treatments are still the most common control tactic used against mealybug pests (Franco *et al.* 2009). Insecticides can be employed to manage these insects with the prerequisite that chemical control is applied during the appropriate period and that the most selective and effective products are used. However, chemical control may often be ineffective, especially when mealybugs reside in hidden locations of the vine (Daane *et al.* 2006). Furthermore, insecticide treatments can negatively impact the natural enemies of the pest (Walton and Pringle 1999). In principal, three main modes of insecticide application are adopted to control mealybugs: foliage cover spraying for management of above-ground populations; application of insecticide solution to the soil to enable it to penetrate to the root zone, so as to combat subterranean colonies; and chemigation by application of systemic compounds via the irrigation system, for example, drip irrigation (Franco *et al.* 2009).

In Tunisia, some insecticides are used against mealybugs in vineyards, such as chlorpyriphos ethyl (480 g/l) at 100 ml/hl and methidathion (400 g/l) at 150 ml/hl. These organophosphates are generally applied during the

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grape-growing season by trunk and foliage spraying, but they have low efficacy on mealybugs. The reason for the low effectiveness against mealybugs is due to the inability of contact insecticides to reach wrapped insects that reside in protected locations within the vine.

More recently, the neonicotinoides, a group of systemic compounds, was found to show high effectiveness against mealybugs and was shown to be safe for other non-targeted organisms (Franco *et al.* 2009). Among these compounds, imidacloprid, for instance, applied through drip irrigation showed high performance in reducing vine mealybug (*P. ficus*) populations and cluster damage related to the latter in California vineyards (Daane *et al.* 2006, 2008).

In the present study, we compared the effectiveness of the systemic insecticide imidacloprid, applied through the drip irrigation system on summer mealybug populations, to methidathion, which is considered to be the most often used insecticide against mealybugs in Tunisian vineyards.

## MATERIALS AND METHODS

### Study site

The study was carried out in 2006 at Beni-Khalled (36°38’58.96”N, 10°35’31.6”E) (North-East region of Tunisia) which had a history of high mealybug infestations. The vineyard cv. Pinot noir has a density of 3 250 vines/ha. It is irrigated with a drip system at a flow rate of 4 l/hour and a watering cycle of 2 days. During the period from the end of April to the end of July, the vines receive between 2 000 and 2 500 $m^3$ of water/ha. The climatic data of Beni-Khalled are indicated in table 1.

### Experimental design

The experimental design was a randomized complete block design with four treatments. Each experimental unit comprised 35 vines and received one of the following treatments: imidacloprid (Spector, Bayer CropScience), at 1 or 2 ml/vine, methidathion (Ultracide 40, Syngenta), at 150 ml/hl, or the untreated control. Insecticide treatments were triggered on June 14, when young (first and second) instar nymphs were abundant on vine trunks, and before the vines had become heavily infested by overlapping mealybug generations.

Methidathion is a non-systemic organophosphate insecticide that inhibits the cholinesterase in the target pests. It should be noted that Methidathion is registered in Tunisia to be used on mealybugs in vineyards, and its application is forbidden during the flowering progress. However, imidacloprid is unregistered for mealybug control in Tunisian vineyards; nevertheless it is registered on meaybugs in Citrus orchards. Imidacloprid is a systemic chloro-nicotinyl insecticide (Placke and Weber 1993). Specifically, it causes a blockage in the nicotinergic neuronal pathway that is more abundant in insects than in warm-blooded animals, making the chemical much more toxic to insects than to warm-blooded animals. This binding on the nicotinic acetylcholine receptor (nAChR) leads to the accumulation of the acetylcholine neurotransmitter, resulting in the paralysis and death of the insect (Okazawa *et al.* 1998). In the present study, imidacloprid was injected through the drip irrigation system to assess the impact of its systemic activity on mealybugs.

### Sampling procedure and evaluation of treatment impact on mealybugs

Treatment efficacy against mealybugs was evaluated on a sample from each experimental unit, which consisted of taking of bark plates from a portion of each vine. Counting of mealybug developmental stages (eggs, first, second, and third instar nymphs, and adult females) was performed with a binocular microscope, at the third and fifth days after treatment application (DAT), then every five days until 60 DAT. The sampled specimens were taken to be mealybugs without indication of species, because it is almost impossible to differentiate, in life, between *P. citri* and *P. ficus* (Williams and Watson 1988; Cox 1989) which often occur together in vineyards of the Cap-Bon region of Tunisia (Mansour 2008; Mansour *et al.* 2009).

Insecticide efficacy on mealybugs was evaluated with the Abbott’s formula:

$$\% \text{ efficacy} = [(T0 - Tt / T0) \times 100]$$

where:
T0 (control) – number of alive mealybugs on untreated vines
Tt – number of alive mealybugs on treated vines

### Statistical analyses

Data related to densities of mealybug developmental stages on all investigated vines were analyzed using the Least Squares Means option in GLM procedure of SAS program (SAS Institute INC., Cary, NC) at 1% probability level.

Table 1. Climatic conditions of Beni-Khalled in 2006

| Months | Jan. | Feb. | Mar. | Apr. | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MT[1] [°C] | 8.8 | 10 | 13.1 | 17 | 21 | 23.2 | 26.2 | 25.7 | 22.5 | 21.3 | 15.3 | 11.8 |
| MP[2] [mm] | 156.9 | 53.5 | 24.3 | 8.5 | 28.3 | 4.7 | 0 | 2.6 | 63.3 | 66.8 | 31.5 | 163.6 |

[1] average temperature; [2] total rainfall

## RESULTS

### Eggs

Three days after treatment (DAT), methidathion sprays induced a decrease of 42.0% on mealybug egg density. However, imidacloprid generated a 66.5% and 76.7% efficacy when applied at 1 and 2 ml, respectively. Ten DAT, an increase in the mealybug reduction ratio (up to 52.6%) was recorded in the methidathion sub-plot. By contrast, 25 DAT, this rate was lower than 50%.

Table 2. Effect of insecticide treatment on mealybug developmental stages on vine

| Treatment | Means of mealybug density/30 investigated vines | | | | |
|---|---|---|---|---|---|
| | adult females | eggs | L1 | L2 | L3 |
| Untreated control | 72.07 a | 359.9 a | 79.6 a | 41.8 a | 103.5 a |
| Methidathion | 29.3 b | 225.6 b | 32.8 b | 33.3 a | 51.6 b |
| Imidaclopride 1 mL | 4.2 c | 85.1 c | 8.8 c | 8.07 b | 12.8 c |
| Imidaclopride 2 mL | 1.5 c | 51.7 c | 2.8 c | 3.5 b | 3.7 c |
| df | 3 | 3 | 3 | 3 | 3 |
| F-value | 19.68 | 12.7 | 7.26 | 13.75 | 5.58 |

Means followed by the same letter are not significantly different at 1% probability level within each column
L1 – first instar nymphs; L2 – second instar nymphs; L3 – third instar nymphs

Imidacloprid applied at 2 ml had an efficacy of 100% on mealybug eggs until 30 DAT. While, applied at a dose of 1 ml, this product provided a maximum efficacy of 86% on mealybug eggs, twenty DAT. By 60 DAT, the efficacy rate remained higher than 50%, no matter what the applied dose had been (Fig. 1).

Statistically speaking, a highly significant ($p < 0.0001$) difference between imidacloprid and methidathion was noted, but no significant difference ($p > 0.01$) was found between the two doses of 1 and 2 ml of imidacloprid (Table 2).

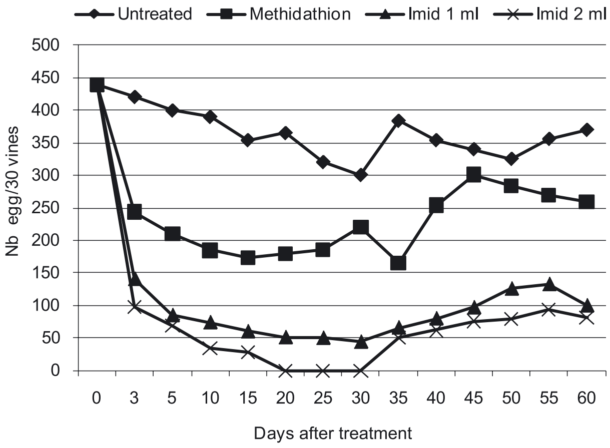


Fig. 1. Mealybug egg density on vine variability overtime

**First instar nymphs**

At 3 DAT, methidathion provided a 63.3% efficacy on mealybug first instar nymphs. A progressive increase in methidathion efficacy was noticed during the twenty DAT (up to 75.5%). Thereafter, this rate decreased, but remained 50% and higher, fifty DAT. These percentages indicate an intermediate effectiveness of methidathion on mealybug first instar nymphs.

Vines treated with imidacloprid had the fewest mealybug egg densities, compared to both methidathion and the untreated control (Fig. 2). Indeed, three DAT, imidacloprid at 1 ml gave a 66.7% efficacy on first instar nymphs, and ten DAT, the rate progressively increased to reach 100%. This rate was maintained during the twenty following days before it decreased at about sixty DAT to a 76.5% efficacy. While, using a 2 ml imidacloprid dose, a 100% rate was maintained longer, for 45 days, with a 88.2% efficacy at sixty DAT. Statistical analyses revealed that vines receiving imidacloprid, regardless of the application dose, had significantly ($p < 0.0001$) less mealybug first instar nymphs than those receiving methidathion (Table 2). There was no significant difference ($p > 0.01$) between the two applied doses of imidacloprid.

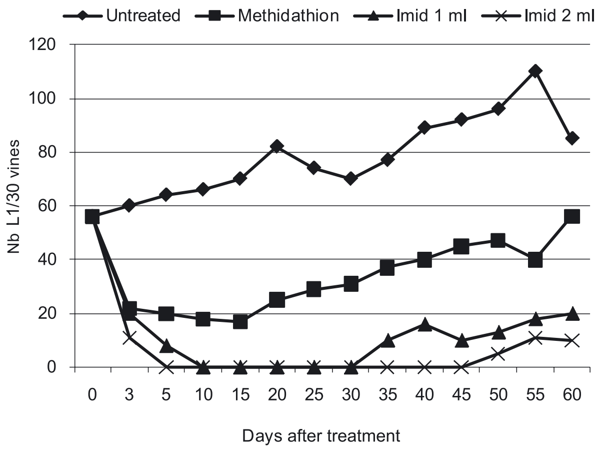


Fig. 2. Mealybug first instar nymph density on vine variability overtime

**Second instar nymphs**

Three DAT, methidathion caused a 14.3% mortality of second instar nymphs, while imidacloprid caused a 71% mortality when applied at 1 ml and 80% when applied at 2 ml. Methidathion did not generate promising results across the whole experiment period, during which its highest effectiveness was recorded three weeks after treatment with a 47.4% mealybug mortality. By contrast, imidacloprid was by far more effective than methidathion on second instar nymphs. Indeed, applied at 1 ml, imidacloprid was 100% effective on mealybug second instar nymphs during the period which extended from 5 to 30 DAT. When applied at 2 ml, this insecticide was 100% effective during the period extending from 5 to 40 DAT (Fig. 3). Until 60 DAT, imidacloprid remained on high performance against second instar nymphs and generated, regardless of the applied dose, a higher than 70% efficacy.

There was no significant difference ($p > 0.01$) between untreated and methidathion treatments (Table 2). However, imidacloprid treatment had significantly ($p < 0.0001$) fewer mealybug second instar nymphs than the untreated control and methidathion. No significant difference ($p > 0.01$) was found between the two applied doses of imidacloprid (Table 2).

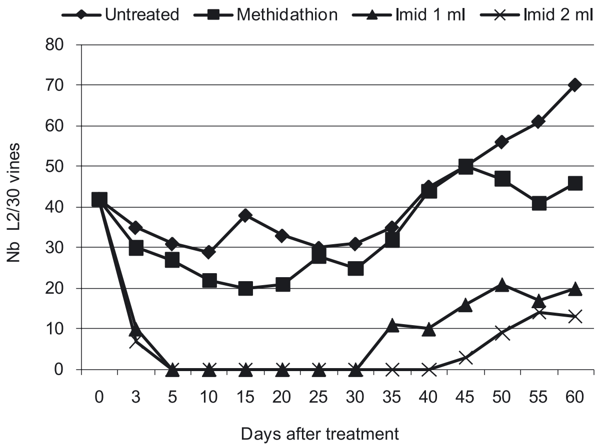


Fig. 3. Mealybug second instar nymph density on vine variability overtime

**Third instar nymphs**

Three DAT, methidathion presented an efficacy of 43.8% on mealybug third instar nymphs. Twenty DAT this rate improved to reach a 69.7% efficacy, before it decreased at about 45 and 50 DAT with a 33.6% and 10% efficacy, respectively. On the other hand, imidacloprid provided promising results at both 1 ml and 2 ml doses with a higher than 70% efficacy during the whole experimental period. Imidacloprid at 2 ml generated a 100% efficacy from the 5th until the 45th DAT, but this efficacy rate was maintained longer when imidacloprid was applied at 1 ml (Fig. 4).

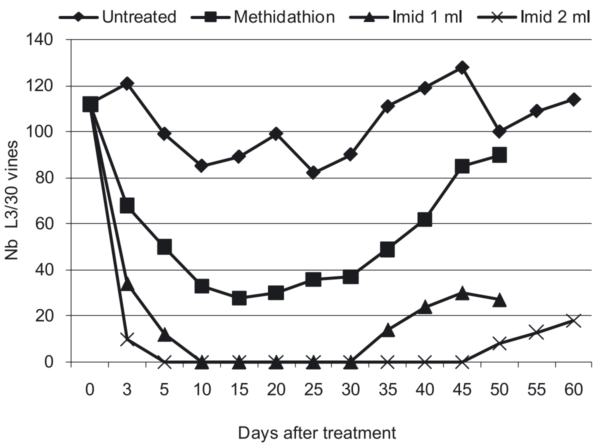


Fig. 4. Mealybug third instar nymph density on vine variability overtime

Statistical analyses showed that the imidacloprid treatment resulted in significant ($p < 0.0001$) suppression of mealybug third instar nymph populations, compared to the methidathion treatment. There was no significant difference ($p > 0.01$) between the applied doses of imidacloprid (Table 2).

**Adult females**

Three DAT, methidathion resulted in a 45% mortality of mealybug adult females. This rate progressively increased to reach 85.7% of effectiveness twenty DAT. Nevertheless, the efficacy rate of this insecticide decreased thereafter with 47.4% at 45 DAT and 11.76% at 60 DAT. By contrast, imidacloprid was by far, better than methidathion in reducing mealybug adult females on vines. Indeed, this systemic insecticide was 100% effective until 35 DAT when applied at 1 ml, and until 45 DAT when applied at 2 ml (Fig. 5). Sixty DAT, imidacloprid was still performing well with an efficacy estimated to be 79.7 and 85.5% for 1 ml and 2 ml applied doses, respectively.

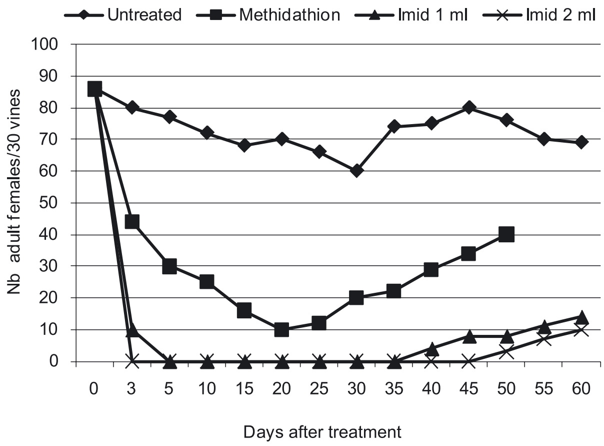


Fig. 5. Mealybug adult female density on vine variability overtime

Statistically, results provided by imidacloprid were significantly ($p < 0.0001$) more satisfactory than those supplied by methidathion based on the reduction of mealybug female densities. No significant difference ($p > 0.01$) was found between the two applied doses of imidacloprid (Table 2).

## DISCUSSION

Abundance of mealybug developmental stages on vine was significantly ($p < 0.0001$) affected by insecticide treatments. However, comparisons of the two imidacloprid treatments to methidathion revealed significant differences based on the reduction of mealybug densities on vines.

Although methidathion appeared to be beneficial for initial mealybug knock-down, it was not sufficiently effective to significantly reduce mealybug population during the post-treatment period. Indeed, this contact insecticide provided average efficacies of less than 40% on eggs, about 60% on first instar nymphs, less than 25% on second instar nymphs, and 50 and 65% on mealybug third instar nymphs and adult females, respectively. According to these findings, it clearly appears that methidathion was more effective on both mealybug first instar nymphs and adult females than on the three other mealybug developmental stages. Furthermore, these results lead us to believe that mealybugs may develop a resistance to methidathion, known as the most used insecticide to control mealybugs in Tunisian vineyards. Flaherty *et al.* (1982) suggested that the multivoltinous character of mealybug pests, and the frequent application of inefficient control measures, accelerate the development of insecticide resistance. In fact, the limited efficacy of methidathion on mealybug populations could be explained by the fact that these insects reside in protected locations and they are protected by their waxy excretion which limits the pest's methidathion uptake. Indeed, Viggiani (1980) stated that the waxy excretion which protects the mealybug's body constitutes a barrier against contact insecticides. Therefore, Mansour (2008) demonstrated that methidathion once applied in summer, provided a low to intermediate overall efficacy on mealybug developmental stages on vine.

On the other hand, the systemic insecticide imidacloprid presented more promising results than those provided by methidathion, based on the reduction of mealybug populations on vines. Thus, imidacloprid, at a dose of 1 or 2 ml/vine, reduced mealybug egg population by 86%, 20 DAT. Therefore, it completely eliminates mealybug's mobile developmental stages from five DAT. The mealybug life stages on vines treated with imidacloprid were missing 40 days after treatment. However, sixty DAT, the efficacy rate, no matter what the imidacloprid applied dose had been, remained higher than 50% for all mealybug life stages. This result is a strong indication that imidacloprid, applied through a drip irrigation system, shows a good long-lasting persistence against mealybug populations on vine within the Cap-Bon Region setting. Daane *et al.* (2006) showed that imidacloprid, applied in a drip irrigation system to control *P. ficus* in California vineyards, provided the greatest reduction in cluster damage, compared to when this product is applied in a furrow-irrigated system. In Tunisia, Mansour (2008) showed that imidacloprid, applied in a furrow-irrigated system, provided low to intermediate effectiveness on mealybug developmental stages on vine. Daane *et al.* (2006) stated that furrow-irrigated blocks have a more widespread root zone than the drip irrigation system. This makes delivery of the insecticide to the entire root zone difficult and results in a more diluted application and poorer uptake of the applied imidacloprid (Daane *et al.* 2006).

The insecticide trial showed that imidacloprid provided an overall high performance in reducing mealybug adult females in the summer period. Thus, it may be useful to use this product in the winter period against overwintering females to prevent the population resurgence of mealybugs in the subsequent generation and to limit further spread in the summer period. The absence of significant differences between the two applied doses (1 and 2 ml/vine) of imidacloprid could incite us to suggest a dose of 1 ml/vine in an IPM strategy. The purpose would be to avoid the presence of even more insecticide residues in grapes.

The obtained results clearly reveal, since mealybugs are both cryptic and sucking pests (phloem feeders), the advantage of the systemic insecticide imidacloprid over the contact insecticide methidathion for the control of mealybug populations on vine. Therefore, imidacloprid through a drip irrigation system could be recommended as a new alternative tool to control mealybugs in Tunisian grape-growing areas. This mode of insecticide application, contrary to the case of a foliar-cover spraying, can be less harmful. This is because it is less likely to come into direct contact with non-target insects such as pollinators (honey bee,…) which can often occur on the crops in the vicinity of vineyards. Felsot *et al.* (1998) suggested that chemigation via subsurface drip irrigation has advantages compatible with environmental stewardship. These advantages include: no worker exposed to foliar pesticide residues, reduction of waste from cleaning out spray tanks, elimination of drift, and less exposure of biological control species to pesticides.

However, in an attempt to enhance mealybug management strategies, other insecticide active ingredients, such as the lipid biosynthesis inhibitor spirotetramat and the insect growth regulator buprofezin, could be tested on these insects in Tunisian vineyards. Additionally, other alternatives to insecticide treatments could, whenever possible, be performed. Among these alternatives, biological control using mealybug parasitoids and mating disruption using female mealybug sex pheromone can prove to be promising tools to effectively control these pests in Tunisian vineyards. For instance, these alternative methods were applied in California vineyards where they contributed to a significant decrease in vine mealybug (*P. ficus*) populations and, hence, limited major economic losses (Daane *et al.* 2006; Walton *et al.* 2006; Daane *et al.* 2008).

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## POLISH SUMMARY

### NAWADNIANIE KROPELKOWE IMIDACHLOPRYDEM OBIECUJĄCĄ ALTERNATYWĄ ZWALCZANIA WEŁNOWCOWATYCH NA PLANTACJACH WINOGRON W TUNEZJI

Wełnowcowate są groźnymi owadami występującymi w uprawach winorośli w Tunezji. Mogą one powodować duże straty w produkcji, dlatego tworzenie programów ich zwalczania jest bardzo ważne, dla plantatorów. Latem, na plantacjach winogron w regionie Cap-Bon, przeprowadzono doświadczenie z użyciem pestycydu. Celem badań była ocena stosowania imidachloprydu – insektycydu systemicznego – przeciwko wełnowcowatym występującym na winoroślach. Imidachlopryd aplikowano drogą nawadniania kropelkowego, a następnie porównano z methidathionem – insektycydem kontaktowym. W zwalczaniu szkodników, we wszystkich stadiach rozwojowych, imidachlopryd był skuteczniejszy niż methidathion, niszczył do 100% owadów. Nie stwierdzono istotnej różnicy pomiędzy dwoma aplikowanymi dawkami imidachloprydu (1 i 2 ml/winorośl). Methidathion wykazywał średnią skuteczność w zwalczaniu szkodników, będąc efektywnym tylko w pierwszym stadium larwalnym oraz stadium dorosłym osobników żeńskich. Stosowanie imidachloprydu drogą nawadniania kropelkowego jest obiecującą metodą zwalczania wełnowcowatych na plantacjach winorośli. Z tego powodu można ją włączyć do programu integrowanej ochrony upraw winorośli w Tunezji.