International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 2 (2): 102-114, 2007
ISSN 1816-4897
© Academic Journals Inc., USA

Adverse Effects of Insecticidal Sprays on Bloom Onset, Pollen Germination and Fruit Set of Three Olive Cultivars

[1]H. Mehri, [2]R. Mehri-Kamoun, [2]A. Ben Dhiab and [2]M. El Mahjoub
[1]Institut National Agronomique de Tunis, 43 Avenue Charles Nicolle, Tunis, Tunisia
[2]Ecole Supérieure d'Horticulture de Chott-Meriem, 4042 Sousse, Tunisia

Abstract: Effects of three insecticides (Dimethoate (Dimate), Deltamethrine (Decis) and Oleoparathion (Oleokriss)) and a bioinsecticide (bactospeine) were compared over three olive cultivars Meski, Gerboui (local) and Coratina (introduced) to determine if insecticide treatment during bloom had an effect on olive productivity. Treatments were applied to field-grown trees during green-cluster stage and their effects were assessed by comparing pollen viability, germination and tube growth, bloom onset and fruit set efficiencies. After spraying, pollen was collected and germinated on insecticide-free media. Individual performance of the three cultivars is illustrated in this study. Generally, all olive cultivars showed similar trends with low pollen germination and tube growth, low fruit set and shortening of flowering period in presence of Oleoparathion and increasing to high levels in presence of Bactopeine. Oleoparathion treatments retarded germination, ruptured pollen tubes and damaged stigmatic surfaces *in vivo*. Germination and tube length were reduced when Dimethoate, Deltamethrine and Oleoparathion at 100% RFR were applied to pollen insecticide-free media. In contrast, at 10 and 1% RFR, these insecticides were less inhibitory and showed no difference from the control. Increased germination percentages and enhanced tube elongation were obtained when Bactospeine was sprayed to trees at 100, 10 and 1% RFR and for all the olive cultivars studied. It increases germination and tube length and has been partially effective in increasing mean fruit set and in shortening fruit development period. Flowering has been delayed for up to five days. Bactospeine sprays increased the extent and earliness of flowering period with increased Bactospeine spray concentration. It advanced blooming by 4-6 days according to cultivar while Oleoparathion shortened it. Bactospeine sprays also resulted in greater fruit set and fruit fresh weights and in earlier fruit ripening compared to the controls.

Key words: Bio-insecticide, insecticide, pollen germination, *Olaea europaea* L., pollen tube morphology

Introduction

Olive is one of the most important crop in Tunisia. Losses from insect pests are important and the olive moth *Prays oleae* (Bernard) is one of the principal insect pests of the olive (Ramos *et al*., 1999). Of its three generations, the anthophagous (flower generation) and carpophagous (fruit generation) are the most damaging. They attack the floral button, reducing the potential number of fruit that can set. The larvae which develop inside the olive stone cause its premature fall reducing productivity (Morris *et al*., 1999).

In Tunisia, olive trees are sprayed with insecticides to prevent and control this plant insects. Many insecticides are used and are targeted specially into the open bloom. Treatment with biological

Corresponding Author: R. Mehri-kamoun, Ecole Supérieure d'Horticulture de Chott-Meriem, 4042 Sousse, Tunisia

insecticide *Bacillus thuringiensis* (Bt) was also used against olive moth *Prays olaea* to reduce the first generation larvae (Jardak *et al*., 1993; Iannotta *et al*., 1998; Mazomenos *et al*., 1999).

Effects of insecticides and particularly bioinsecticide applied during bloom period on pollen capacity (viability, germination and tube elongation) are little known. Many researchers noted the fungicide efficacy but only a few reports documented the effect of insecticides on tree physiology and productivity (Rosenberg *et al*., 2003). The recent one report was of Mehri *et al*. (2006) on olive pollen. The effect of three insecticides (Dimethoate (Dimate), Deltamethrine (Decis) and Oleoparathion (Oleokriss)) and a bio-insecticide (bactospeine) at three concentrations (100, 10 and 1% recommended field rate) on pollen germination and tube growth rates were investigated using *in vitro* culture of pollen collected from three olive cultivars Gerboui, Coratina and Meski. By *in vitro* assays, the insecticides (Dimethoate, Deltamethrine, Oleoparathion) have been reported to inhibit pollen germination and tube growth according to the concentration and the product used. In contrast, increased germination percentages and enhanced tube elongation were obtained in presence of Bactospeine for all olive cultivars studied.

The objective of this study were to evaluate the effectiveness of three insecticides and a bioinsecticide applied to shoots grown in the field and sprayed at three concentrations (100, 10 and 1% recommended field rate) on pollen capacity (viability, germination and tube growth), bloom onset and fruit set of three olive cultivars Meski, Gerboui (local) and Coratina (introduced).

Materials and Methods

Three trees of each cultivar were used for these essays. Three insecticides included in comparisons were (Dimethoate, Deltamethrine, Oleoparathion) and a microbial control agent (Bactospeine) that were applied to olive trees during the blooming season. The class, trade name, formulation, active ingredient and recommended field rate for the four compounds are summarized in Table 1. For each cultivar, pollen viability, pollen capacity (germination and tube growth), length of bloom onset and fruit set were assessed at three concentrations of the compounds: 100% the recommended field rate RFR, 10 and 1% RFR. This study was conducted in the region of Chott-Meriem (central part of Tunisia) during 2003.

The experimental design was a randomized complete block with four treatments using three-tree plots. All test plots were located in a single row through the center of the orchard. All sprays were applied at 3 concentrations using a conventional sprayer delivering 400 L ha^{-1} about 15-18 L/tree. Individual shoots of uniform diameter and length containing inflorescences were sprayed once in the end of April (green cluster stage). Insecticide and bio-insecticide sprays and control were with three shoots in treatment. The controls, the shoots were sprayed with distilled water.

The plants were at the green cluster stage at the time of spray applications. 3 replicates per treatment were used. The three plants of each variety were used as three experimental blocks, selected for their uniform size and vigour with high flowering load. All trees received identical management practices (fertilizing, watering, etc.).

Table 1: Selected insecticides and their Recommended Field Rates (RFR)

Active ingredient	Trade name	Formulation	Active ingredient (%)	RFR
Bacillus thuringiensis	Bactospeine 16000	WP	16000 UI mg^{-1}	100 g hl^{-1}
Deltamethrine	Decis EC 25	EC	25 g L^{-1}	200 cc hl^{-1}
Dimethoate	Dimate	EC	40%	100 cc hl^{-1}
Oleoparathion	Oleokriss	EC	Parathion Ethyl 3%	750 cc hl^{-1}

EC: Emulsifiable Concentrate, WP: Wettable Powder

Flower Morphology

The phenologic status of productivity for the three cultivars treated with insecticides and with distilled water were recorded. Fifteen bearing shoots per cultivar were tagged and the number of flowers per inflorescence, of staminate and perfect flowers per inflorescence were counted at anthesis. Flowering periods were determined on the basis of phenological stages when 10% (beginning of flowering) and 50% (full bloom) of flowers were opened and petals had fallen (end of flowering). Flower morphology was determined by examining 50 flowers of each cultivar collected at random throughout the tree, at the beginning of bloom.

Pollen Viability and Capacity

Inflorescences of treated shoots and control were collected at white-button stage. Anthers were taken off and allowed to dry in the laboratory, they were dehisced overnight at room temperature and pollen collected. Pollen grains were cultured on free insecticide medium with three replications per treatment. Pollen from shoots sprayed with distilled water and germinated in free-insecticide medium served as control.

Pollen viability, germination and tube elongation abilities of all the cultivars were tested. A comparison of pollen viability (abnormal pollen grains) of all the cultivars was done using acetocarmin staining technique (Martoja and Martoja-Pierson, 1967).

Pollen grains from treated and untreated flowers were cultured on a sucrose-agar medium containing 100 ppm H_3BO_3 (Mehri *et al*., 2003) at pH 5. Cultures were held at 25°C for 48 h. Counted pollen grains were at least 500 per petri dish. A pollen grain was considered germinated when the length of its pollen tube was equal to or exceeded its diameter (Stanley and Linkens, 1974). Pollen germination and tube growth were recorded from 4 to 48 h of incubation, on pollen grains chosen at random from various locations in the pollen sample. Germination rate was determined using five replicates of approximately 100 grains (Pinney and Polito, 1990).

Fruit Set

To analyze the stimulatory or inhibitory effects of insecticides on olive fruit percentage, fruits from each cultivar were selected at 45 days after full bloom (FB) (initial fruit set) and at harvest (final fruit set). Effects of treatments on fruit set were determined by counting the number of pollinated fruitlets on about 100 inflorescences per tree. Measurements of fruit length and weight were also recorded.

Statistical Analysis

A factorial treatment was used in this study. Each treatment (3 insecticides + 1 bio-pesticide × 3 concentrations with control) on three olive cultivars Coratina, Gerboui and Meski. The effect of insecticides on germination and pollen tube length, bloom onset and fruit-set for three olive cultivars was analyzed by analysis of variance. Student-Newman-Keuls test was used to compare the treatments.

Results

Germination and Tube Growth

Control

The mean germination percentage in the control (when pollen exposed to the water spray was incubated in insecticide-free media) and after 4 h incubation was 18.6, 24.5 and 33.5%, respectively for Meski, Gerboui and Coratina cultivars. The percentage viability of pollen grains was high 96.3,

Table 2: Pollen viability percentage of three olive cultivars Meski, Coratina, Gerboui

		Cultivars		
Treatments	Concentration	Meski	Gerboui	Coratina
Control	-	96.32±3.1%	91.26±4.3%	93.22±2.31%
Bactospeine	100% RFR	96.96±2.4%	92.31±3.1%	94.82±1.92%
	10% RFR	96.46±2.1%	91.55±2.5%	93.81±3.01%
	1% RFR	96.28±1.1%	91.32±5.01%	93.62±1.6%
Oleoparathion	100% RFR	94.32±3.3%	89.9±2.8%	92.33±2.41%
	10% RFR	94.12±2.0%	90.92±2.4%	92.98±2.72%
	1% RFR	95.22±1.2%	90.99±1.7%	93.12±3.4%
Deltamethrine	100% RFR	96.23±1.7%	91.01±1.91%	93.08±1.32%
	10% RFR	95.62±0.9%	90.32±3.1%	93.18±1.90%
	1% RFR	96.12±1.9%	90.82±1.87%	93.02±2.01%
Dimethoate	100% RFR	95.17±2.05%	91.21±2.01%	93.03±2.11%
	10% RFR	95.22±1.1%	91.12±1.61%	93.06±1.61%
	1% RFR	95.42±2.1%	91.88±1.12%	93.17±1.47%

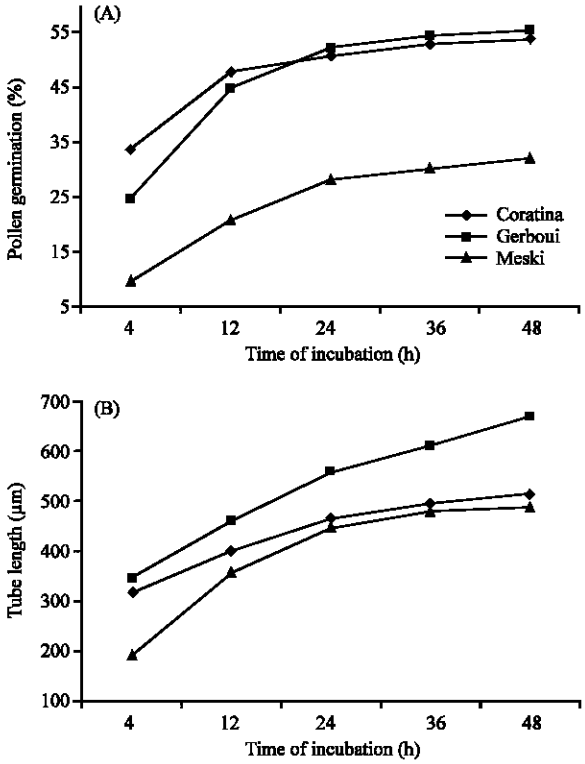

Fig. 1: Percentage of freshly harvested pollen of three olive cultivars Meski, Gerboui and Coratina, cultivated after 4, 12, 24 and 48 h in sucrose-agar medium containing 100 ppm H_3BO_3, at 25°C in dark: (A) Germination percentage of pollen grains, (B) pollen tube length. Data are the means of 100 grains

91.26 and 93.2% for Meski, Gerboui and Coratina cultivars, respectively (Table 2). After 48 h incubation, the germination rate of pollen exposed to the distilled water (control) average 32% in Meski, 55% in Gerboui and 54% in Coratina cultivar (Fig. 1A). Figure 1B indicates that pollen tube length was significantly higher for Coratina (668 µm) than Meski and Gerboui (514 and 488 µm), respectively.

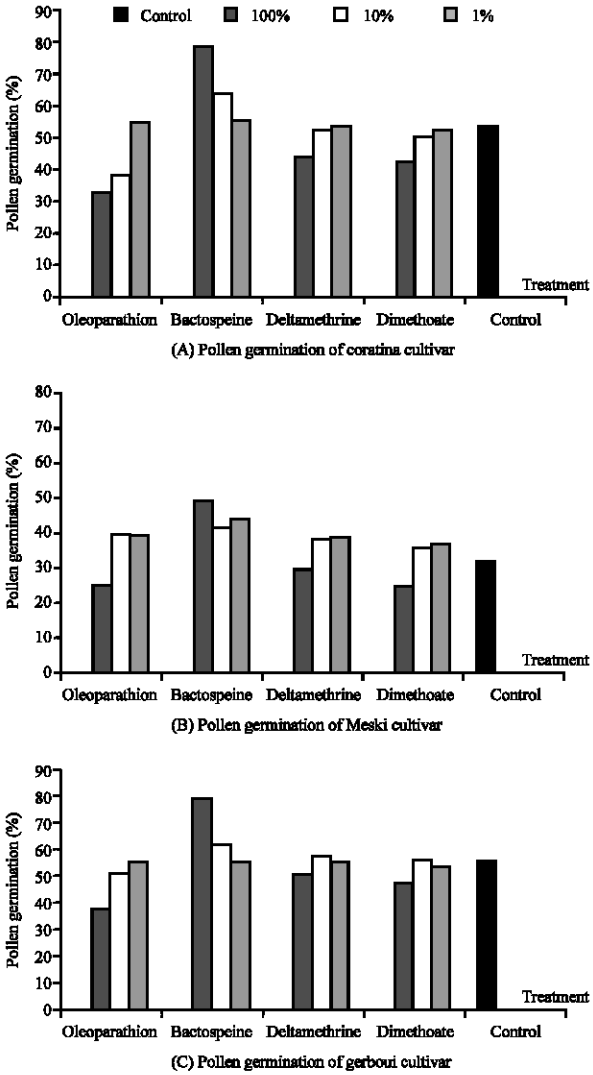

Fig. 2: Effect of three insecticides and a bio-insecticide sprayed before bloom on pollen germination percentage of three olive cultivars. Pollen cultivate on sucrose-agar medium containing 100 ppm H_3BO_3 at pH = 5 and in dark

Treatments

The variety × treatment interaction was significant indicating that cultivars differed in their response to the insecticide treatment. We noted cultivar differences in the reaction of olive pollen, flowering and fruit-set to insecticide sprays. Individual performance of the three cultivars is illustrated in Fig. (2, 3 and 5).

No noticeable differences were found among pollen grain viability of the three olive cultivars studied from the insecticide concentrations of 100, 10 and 1% RFR (Table 2) but there were significant effects of cultivars, insecticide treatments and concentration on pollen germination and tube elongation (Fig. 2).

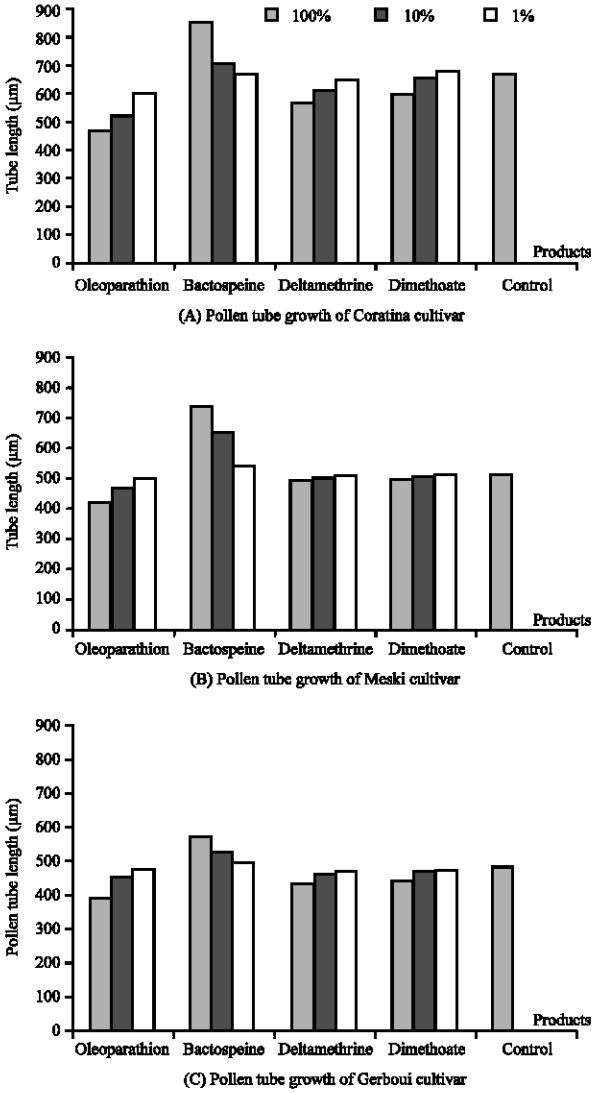

Fig. 3: Effect of three insecticides and a bio-insecticide sprayed before bloom at three concentrations 100, 10 and 1% RFR on pollen tube growth of three olive cultivars. Pollen cultivate on sucrose-agar medium containing 100 ppm H_3BO_3 at pH = 5 and in dark

Dimethoate, Deltamethrine and Oleoparathion at 100% RFR significantly reduced pollen germination and tube elongation compared with water controls. In contrast Bactospeine at 100 and 10% increased germination and enhanced pollen tube growth for all the cultivars tested (Fig. 2 and 3).

In all the cultivars studied, there were no significant differences in mean pollen germination percentage and tube growth among control (water sprayed) and the three insecticide flower sprayed at 10 and 1% RFR (Fig. 2 and 3). In control (pollen from inflorescences sprayed with water), the mean germination percentage were significantly greater than in Oleoparathion, Deltamethrine and Dimethoate at 100% RFR for all the cultivars. While the treatments with Bactospeine sprayed at 100, 10 and 1% were higher than control (Fig. 2).

Effects of the Cultivars

In Coratina (Fig. 2A), there were no significant differences in mean per cent pollen germination among control (water spray) and 1 and 10% RFR Deltamethrine and Dimethoate sprayed inflorescences. Oleoparathion sprays at 100% RFR significantly decreased germination in comparison with the control and resulted in a further decrease in comparison to Deltamethrine and Dimethoate at 100% RFR.

In Meski (Fig. 2B), no significant effect on mean per cent pollen germination was found among Oleoparathion, Deltamethrine and Dimethoate at 1 and 10% RFR and the water sprayed (control). These insecticide treatments at 100% RFR significantly decreased pollen germination in comparison with all other treatments except the water spray.

In Gerboui (Fig. 2C), the same behaviour can be deduced from the results obtained among control and 1 and 10% RFR insecticides but the lowest mean percentage germination was observed in Oleoparathion at 100% RFR. Treatments at 100% significantly reduced pollen germination in comparison with pollen from either Deltamethrine and Dimethoate treatment.

Effects of the Insecticides

Oleoparathion

In general Oleoparathion that had the greatest inhibition on pollen germination also had the greatest inhibitory effects on tube growth. Among the insecticide treatments, Oleoparathion at 100% RFR had the greatest inhibitory effects on pollen germination. Pollen collected from shoots which received Oleoparathion sprays made less extensive tube growth that controls. Oleoparathion (Fig. 3A) sprayed at 100% RFR resulted in the least germination after 48 h incubation in Meski cultivar (15.2%) followed by Coratina (33.1%) and Gerboui (37.4%). At 1% Oleoparathion, no significant effect on mean percent pollen germination was found between control and cultivars. With this insecticide and for all the olive studied here, the percentage pollen germination after 48 h incubation was higher than after 4 h incubation but the increase was slight.

Compared with controls, Oleoparathion and Deltamethrine resulted in pollen with more abnormal morphology. A higher proportion of abnormal grains were observed in 100% RFR of the 2 insecticides. There were from 6 at 100% RFR Deltamethrine to 24 at 100% Oleoparathion. These abnormal pollen were often smaller and aggregated into clumps and collapsed at polar ends. They are irregularly shaped and were empty as lacking cytoplasmic components (Fig. 4). In addition to inhibiting pollen germination and tube elongation, Mehri *et al.* (2006) showed that the few pollen germinated when these insecticides were applied in culture media, exhibited abnormal growth of pollen tube. Some pollen tubes were characterized by swelling and rupture in the tip region with a sinuous and wavy configuration. Jaycox and Owen (1965) suggested the possibility of physical action of sprays damaging pollen grains.

Dimethoate and Deltamethrine

Sprayed at green cluster stage, Dimethoate and Deltamethrine at 100% RFR reduced germination and tube growth but less severely decreased than with Oleoparathion. At 10 and 1% RFR, germination percentage and tube elongation were not significantly different from those of the control. Intermediate inhibitory effects on tube length were observed with Dimethoate and Deltamethrine on Meski and Gerboui cultivars. Tube elongation was no different from controls.

Field application of these insecticides on olive have inhibited pollen germination and tube growth both *in vitro* and *in vivo* assays but not equally for the three olive cultivars studied in this work. Also contrasts between various insecticide groups showed different results. Bactospeine is a *Bacillus thuringiensis* (Bt) used for controlling pests, is a most widely used microbial control agent and serve as alternatives to broad spectrum chemical insecticide (Lacey *et al*., 2001). It is a delta-endoxin which exhibit larvicidal toxicity upon ingestion lepidopterean and dipterian larvae (Zouari *et al*., 2000).

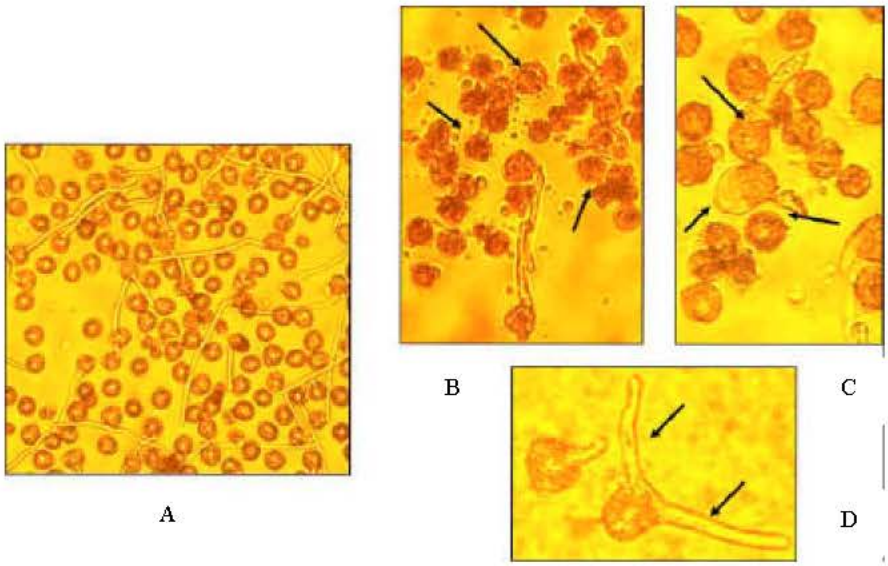

Fig. 4: Germination and tube morphology of olive pollen exposed to insecticide sprays and incubated in insecticide-free media: (A) pollen exposed to bactospeine showing straight and long tubes, (B, C and D) pollen exposed to Oleoparathion sprays showing a high proportion of abnormal grains which are small and aggregated into clumbs and collapsed at polar ends. Arrows indicate grains irregularly shaped and empty as lacking cytoplasmic components (C) and abnormal tube growth (D)

Civantos and Sanchez (1993) used Bt as integrated control in Spanish olive groves and concluded that the results were similar to those with organophosphate insecticides. Bt is a prevalent organism on wine grapes where it has the potential to control insects and also to control spoilage and mycotoxigenic fungi on grapes and to influence the phyllospheric microflora (Bae *et al*., 2004). The efficacy of Bt has been evaluated against olive pyralid (*Palpita unionalis* Hubner), it reduced the population if P. larvae on shoots and was more effective if applied twice and sprayings are spaced at 7-10 days on olive trees (Albanese *et al*., 2000).

Dimethoate is an organophosphate and a very toxic systemic insecticide which kills insects by interfering with the action of important enzymes in the nervous system has been used as larvicide treatment against olive fly (*Bactrocera oleae* Gmel) (Laccone *et al*., 2000; De Nino *et al*., 2000). It resulted in successful control of the pest and it is no detected in olive and oil samples tested after analyzing the active substances (Parlatti *et al*., 2000).

Deltamethrine is a pyrethoid insecticide used to control *Phloeotribus scarabaeoides* Bern. in olive orchards. It has a negative effect on different parasitoid families and especially parasitoids of *Prays oleae*, the major pest of olive trees (Rodriguez *et al*., 2003).

Many insecticides have been used against *Prays oleae* but the only effective methods of control available against carpophagous generation are systemic chemicals that are highly polluting to the environment and the product and their use is not always justified (Baker, 1982; Iannotta *et al*., 1998). Many of beneficial insects present in the olive orchards are seriously affected by conventional treatments (Campos and Civantos, 2000) and their increased utilization will require increased pathogen virulence (Lacey *et al*., 2001). The use of insecticides has created environments which have favored the development of resistance in many species that have been exposed to them. It may bring about a

Table 3: The onset of the bloom period of three olive cultivars sprayed with insecticides at three concentrations

Treatments	Concentrations	Meski	Gerboui	Coratina
Control	-	34 days	24 days	30 days
Bactospeine	100% RFR	37	29	30
	10% RFR	31	25	32
	1% RFR	41	24	30
Oleoparathion	100% RFR	23	16	24
	10% RFR	28	22	23
	1% RFR	33	23	28
Deltamethrine	100% RFR	30	21	27
	10% RFR	33	23	28
	1% RFR	34	23	30
Dimethoate	100% RFR	30	22	25
	10% RFR	33	21	26
	1% RFR	32	23	28

concomitant erosion of genetic variability. In contrast, microbial control agents such as Bactospeine serve as alternatives to broad spectrum chemical insecticide. Scientists fear their long-term effectiveness will be threatened by the development of Bt-resistant strains (Lacey *et al*., 2001).

Flower Onset

In the control, the onset of the bloom season varied according to the cultivar between 35 days for Meski, 24 days for Gerboui and 29 days for Coratina. The influence of insecticides (type and concentration) on flower emergence depends on the blooming stage of each cultivar. Oleoparathion at 100 and 10% RFR reduced significantly the length of blooming period of all the cultivars studied. The same behaviour can be deduced when Deltamethrine and Dimethoate at 100 and 10% RFR were applied but a slight effect. In contrast, Bactospeine sprays at 100 and 10% RFR increase the length of bloom period (Table 3) and the extent and earliness of flowering of the three olive cultivars. However, interactions between spray concentrations and cultivar were observed. When shoots were sprayed with Bactospeine, the blooming length increased with increased Bactospeine spray concentration. The length of blooming period of Meski cultivar was 41 and 37 days from treatment at 100 and 10% RFR compared to the control (34 days). By 20 and 25 April, 10% of the flowers treated were opened for the 100 and 10% Bactospeine sprays compared with the date of 1st May for the controls. At 1%, they did not reflect any significant effect. This extension of the bloom period can be explained by the opening of few flowers during the last days of the bloom which prolongs the blooming season. The insecticides may reduce the number of opening flowers. For the three olive cultivars studied, Bactospeine spray concentrations resulted in earlier blooming compared to the controls.

These findings showing the fast effect of insecticides on the length of blooming period, is critical of pollination and for ensuring a crop in commercial orchards.

Fruit Set

Fruit set percentage was strongly affected and influenced by insecticide sprays at 100 and 10% RFR for all olive cultivars studied. Dimethoate, Deltamethrine and Oleoparathion at 100% RFR significantly reduced fruit-set of Meski, Coratina and Gerboui cultivars. The greatest reduction occurred when Oleoparathion was sprayed and the fruit set was increased by the decrease of concentration from 100% RFR to 1% RFR, it was similar to the control. There were no significant differences in fruit set in either 2 concentrations for 10 and 1% RFR compared to the control in Gerboui cultivar but in Meski and Coratina cultivars, mean fruit set was higher at 1% than at 10%

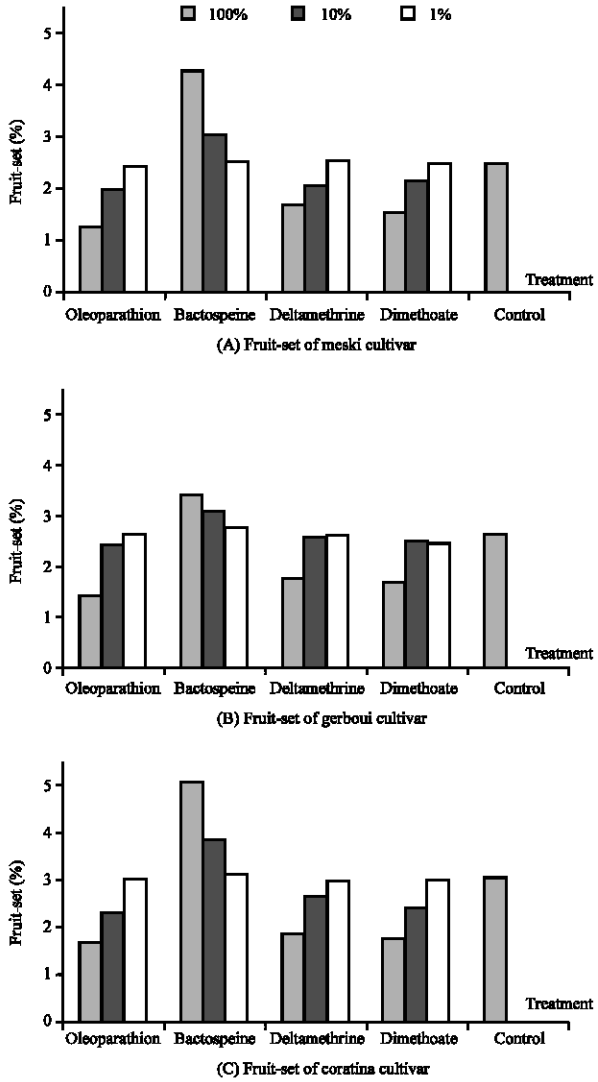

Fig. 5: Percentage fruit-set of three olive cultivars after spraying three insecticides and a bio-insecticide at three concentrations 100, 10 and 1% RFR

(Fig. 5 A, C). All insecticide treatments reduced fruit set but the greatest increase occurred with Bactospeine treatment at 100% RFR and 10% RFR for all the cultivars.

In Gerboui cultivar, these insecticides did not affect the fruit set percentage when applied at 10 and 1% RFR while in Coratina and Meski, fruit set is higher with the decrease of concentration from 10 to 1% RFR (Fig. 5B). At 100 and 10% RFR, Bactospeine increased germination and tube growth and has been effective in increasing mean fruit set and fruit size.

There were a significant reduction in fruit size when the insecticide treatments were applied. Fresh weight and fruit length/diameter ratio (L/D) were increased by Bactospeine sprays while Oleoparathion application caused the greatest reduction and we observed fruits irregularly shaped and asymmetrical (Fig. 6).

Fig. 6: Effect of an insecticide (Oleoparathion) and a bio-insecticide (Bactospeine) sprays during bloom on fruit size and fruit length/diameter ratio of olive cultivar meski compared to the control

Discussion

Chemical treatments remains one of the most important management activities in olive production and many insecticides are currently registered in Tunisia for controlling many olive pests. In the current study, three insecticides and a bio-insecticide at three concentrations, are sprayed at green-cluster stage of three olive cultivars and were evaluated to compare their effectiveness on pollen capacity (viability, germination and tube growth), flowering and fruit set. Individual performance of the three cultivars is illustrated in this study. Generally, all olive cultivars showed similar trends with low pollen germination and tube growth, low fruit set and delay of flowering period in presence of Oleoparathion and increasing to high levels with Bactopeine treatment. Oleoparathion treatments retarded germination, ruptured pollen tubes and damaged stigmatic surfaces *in vivo*. Germination and tube length of pollen were reduced when Dimethoate, Deltamethrine and Oleoparathion at 100% RFR were sprayed in the field. In contrast, at 10 and 1% RFR, these insecticides were less inhibitory. An intermediate inhibitory effect on pollen germination and tube elongation were observed when Dimethoate was used. Bactospeine was the earliest to bloom and Oleoparathion was consistently been the latest blooming olive. The bloom season was early compared to average blooming dates. Bactospeine has shown positive effects that have commercial potential on olive. These include increased fruit weight, fruit set, extension of full bloom, increased pollen germination and tube growth. It should be evaluated at different period.

The apparent effect of Bactospeine spray was to prevent successful fertilization by the pollen which had already germinating during the 24 h of incubation. It increased germination and tube length and has been partially effective in increasing mean fruit set and in shortening fruit development period. Flowering has been delayed for up to five days. Bactospeine sprays increased the extent and earliness of flowering period with increased Bactospeine spray concentration. It advanced blooming by 4-6 days according to cultivar. Full bloom date was strongly affected by Dimethoate, Deltamethrine and Oleoparathion treatments and flowering period was shortened by 8-10 days leading to floral fading and consequently reduced fruit setting. Also considerable stigmate necrosis was observed at the 100% insecticide treatment mainly with Oleoparathion.

In general, Bactospeine advanced blooming by 4 to 9 days according to the cultivar while Oleoparathion shortened it. Bactospeine sprays also resulted in greater fruit set and fruit fresh weights and in earlier fruit ripening compared to the controls. Mean fruit weight increased as Bactospeine spray concentrations increased from 1 to 100% RFR. Flower bud thinning in Bactospeine treatments may explain the additional increase in early flower period and greater mean fruit fresh weight obtained from that treatment. The economic impact of this aspect is very significant because this product not only reduced the economic losses due to the reduce of pest attack but also its secondary effects on the beneficial fauna (Ramos *et al*., 1999; Bae *et al*., 2004).

The fast activity of insecticides treatment *in vivo* (sprayed on shoots during green-cluster stage and incubated on insecticide-free medium) reported in this investigation was confirmed by our same investigation under *in vitro* conditions (insecticides were applied in germination medium) but rates *in vivo* were lower. These insecticides inhibited pollen germination when added to the culture medium (Mehri *et al*., 2003) or sprayed on the clusters just before blooming, although the effect was higher *in vitro* than *in vivo*. It suggested that the undehisced anther wall may protect pollen from contact with insecticide sprayed at green-cluster stage. The results suggest that *in vitro*, the toxic effect of insecticides appear during germination, resulting in the pollen is arrested in its development by direct contact with insecticide. The values are different when these insecticides were applied on shoot just before blooming and when applied in the pollen culture media.

In addition to decreased germination rates, pollen collected from shoots which received insecticide sprays produced shorter tubes than control. Patterns of pollen tube growth differed among the type and concentration of insecticides and among the cultivars. The decrease in pollen capacity associated with insecticidal sprays has adversely affect fertilization and subsequent fruit set, it significantly altered pollen morphology. Further investigations to consider must include effects on pollination. He and Wetzstein (1994) noted that no differences in pollen tube growth but ovule longevity showed early signs of degeneration and senescence.

Yield was not measured but the observations indicated that fruit set and fruit weight were greater and the blooming date was advanced by several days when the bio-insecticide was applied at the green cluster stage but additional work is need to explore the interactions of this product with insecticidal sprays.

The carry-over effects of the three insecticides and the bioinsecticide on vegetative and reproductive behavior of each olive cultivar the season following application need further investigation. It includes effects on plant growth, pollen development and pollination to select the less damaging pesticides for use at or near pollination in olive trees.

References

Albanese, M., C. Cafueri, G. Defeudis, G. Grosso, S. Del and I. Digermanio, 2000. Evidence of control with biological products against the margaronia or the olive pyralid (*Palpita unionalis* Hubner.). Atti Giornate fitotatologiche, Perugia, 1: 395-400.

Bae, S., G.H. Fleet and G.M. Heard, 2004. Occurrence and significance of *Bacillus thuringiensis* on wine grapes. Intl. J. Food Microbiol., 94: 301-312.

Baker, J., 1982. Selective effects of insecticides on within-species variation, the lessons to be learnt when considering the environmental effects of polluants. Agric. Environ., 7: 187-198.

Campos, M. and M. Civantos, 2000. Technicas de olivo y su incidencia sobre las plagas. Olivae, 84: 40-46.

Civantos, M. and M. Sanchez, 1993. Integrated control in Spanish olive groves and its influence on quality. Agricultura, Revista Revista Agropecuaria, 62: 854-859.

De Nino, A., A. Procopio, A. Ruffolo, A. Tagarelli, G. Sindonia and E. Perri, 2000. Analysis of Pesticide Residues in Olive Oil Using Labelled Internal Standard. Abstract in 4th International Symposium on Olive Growing, ISHI, Acta Hortic. 586, Vol. 1-2. Eds. Vitagliano, C., G.P. Martelli, Valenzano, Italy.

He, Y. and H.Y. Wetzstein, 1994. Pollen degeneration and retarded leaf development from fungicidal sprays applied during microspore development and shoot expansion. J. Hortic. Sci., 69: 975-983.

Iannotta, N., G. Giordano and G. Rende, 1998. The olive moth in Calabria. Informatore Agrario, 54: 69-73.

Jardak, T., M. Ksantini and M. Moalla, 1993. Essais de traitement à l'aide de *Bacillus thuringiensis Berliner:* Formule en crème et en poudre mouillable pour lutter contre la première génération de *Prays oleae* Bern. Institut de l'olivier, Note technique, 1/93, pp: 7.

Jaycox, E.R. and F.W. Owen, 1965. A pollination experiment using honeybees and pollen insects to improve fruit setting in a low yield orchard. Trans. Ill. St. Hortic. Sci., 98: 102-107.

Laccone, G., R. Falco, G. Milella, C. Nasole, G. Papa and M. Sorrenti, 2000. Efficacy, persistence and phytotoxicity of dimethoate, phosphamidon, formothion in controlling *Bactrocera oleae Gmel.* on Coratina oil olives in Apulia, Southern Italy. Atti, Giornate Fitopatologiche, Perugia, 1: 401-406.

Lacey, L.A., R. Frutos, H.K. Kaya and P. Vail, 2001. Insect pathogens as biological control agents: Do they have a future? Biol. Control, 21: 230-248.

Martoja, R. and M. Martoja-Pierson, 1967. Initiation aux techniques de l'histologie animale. Edition Masson et Cie, Paris 4: pp: 330.

Mazomenos, B.E., A. Ortiz, A. Mazomenos-PantazI, D. StefanoU, N. Stavrakis, C. Karapati and M. Fountoulakis, 1999. Mating disruption for the control of the olive moth, *Prays oleae* (*Bern*) (*Lep*., *Yponomeutidae*) with the major sex pheromone component. J. Applied Entomol., 123: 247-254.

Mehri, H., R. Mehri-Kamoun, M. Msallem, A. Faidi and V. Polts, 2003. Reproductive behaviour of six olive cultivars as pollenizer of the self-incompatible olive cultivar Meski. Adv. Hortic. Sci., 17: 42-46.

Mehri, H., R. Mehri-Kamoun, A. Ben Dhiab and M. El Mahjoub, 2006. The effect of bactospeine and three insecticides on olive pollen germination and tube growth. Adv. Hortic. Sci., 20: 140-146.

Morris, T.I., M. Campos and N.A.C. Kidd, 1999. What is consuming *Prays oleae* (Bernard) and when a serological solution. Crop Protec., 18: 173-192.

Parlatti, M.V., S. Pandolfl, A. Leandri, V. Pompi and L. Forchielli, 2000. Control of *Bactrocera oleae* (Gmel) and persistence of some insecticides in olives and in oil. Atti, Giornate Fitopatologiche, Perugia, 1: 181-188.

Pinney, K. and V.S. Polito, 1990. Olive pollen storage and *in vitro* germination. Acta Hortic. 286: 207-210.

Ramos, P., M. Campos and J.M. Ramos, 1999. Long-term study on the evaluation of yield and economic losses caused by *Prays oleae* Bern. in the olive crop of Granada (southern Spain). Crop Protec., 17: 645-647.

Rodriguez, E., A. Pena, A.J. Sanchez Raya and M. Campos, 2003. Evaluation of the effect on arthropod populations by using deltamethrin to control *Phloeotribus scarabaeoides* Bern. (Coleoptera: Scolytidae) in olive orchards. Chemosphere, 52: 127-134.

Rosenberg, D.A., T.L. Robinson and F.W. Meyer, 2003. Effect of sterol-demethylation inhibiting fungicides on apple fruit-set, fruit size, total yield and gross returns. Hortscience, 38: 601-604.

Stanley, R.G. and H.F. Linkens, 1974. Pollen Biology, Biochemistry, Management. Springer-Verlag, Berlin, pp: 211.

Zouari, N., S. Ben Sikali and S. Joua, 2002. Production of delta-endoxins by *Bacillus thuringiensis* strains exhibiting various insecticidal activities towards lepidoptera and diptera in gruel and fish meal media. Enzyme Microbiol. Technol., 31: 411-418.