Lethal and Sublethal Effects on *Tamarixia triozae* (Hymenoptera: Eulophidae), an Ectoparasitoid of *Bactericera cockerelli* (Hemiptera: Triozidae), of Three Insecticides Used on Solanaceous Crops

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Abstract

Lethal and sublethal effects of refined soybean oil, imidacloprid, and abamectin on *Tamarixia triozae* (Burks; Hymenoptera: Eulophidae) were assessed after exposure of the eggs, larvae, and pupae of this parasitoid to three concentrations of these active substances: the LC₅₀ for fourth-instar *Bactericera cockerelli* (Sulc.; Hemiptera:Triozidae) and 50% and 100% of the minimum field-registered concentration (MiFRC). Soybean oil caused 26–61% mortality in *T. triozae* eggs and 6–19% in larvae; mortality in both eggs and larvae was ≤19% for imidacloprid and 4–100% for abamectin. All three compounds caused <18% mortality of *T. triozae* pupae, with the exception of the abamectin 50% (47%) and 100% (72%) MiFRC. The mortality of larvae and pupae derived from treated eggs was ≤39% for all three insecticides, and that of pupae derived from treated larvae was ≤10%. In general, emergence of adults developed from treated eggs, larvae, and pupae was affected more by abamectin than by the other treatments. The proportion of females derived from all three development stages was not affected by treatment with the compounds, except when the parasitoid was treated as larvae with the soybean oil 100 and 50% MiFRC (66 and 68%, respectively) or when treated as pupae with the imidacloprid LC₅₀ and 100% MiFRC (~60%). Female longevity was generally higher than that of males. The use of imidacloprid, soybean oil, and abamectin in combination with *T. triozae* for pest control may be effective when the parasitoid is in the pupal stage because this stage is less susceptible than other immature stages.

Key words: synovigenic ectoparasitoid, biological control, life parameter, tomato/potato psyllid

The tomato or potato psyllid *Bactericera cockerelli* (Sulc.; Hemiptera: Triozidae) is a highly polyphagous insect that feeds on more than 40 plant species from over 20 families but shows a strong preference for certain solanaceous crops (Yang et al. 2010). This species is the principal vector of the bacterium *Candidatus Liberibacter solanacearum* (a.k.a. Ca. Liberibacter psyllaurous), which is responsible for zebra chip (ZC) disease in potato (*Solanum tuberosum* L.) (Munyaneza et al. 2007), tomato (*Solanum lycopersicum* L.) (Munyaneza and Sengoda 2009), and pepper (*Capsicum annuum* L.) (Munyaneza and Sengoda 2009) crops, all three species (Solanales: Solanaceae).

In Mexico, control of *B. cockerelli* populations has principally been realized through the use of synthetic insecticides belonging to the carbamate, organophosphate, pyrethroid, and spinosyn groups (Dávila et al. 2012), though other compounds such as imidacloprid, thiamethoxam, abamectin, spirotetramat, spiromesifen, and pyriproxyfen are also occasionally applied. However, the intensive use of pesticides has compromised their effectiveness, especially due to the development of resistance in the pest to many of the main active substances available on the market (Dávila et al. 2012). To avoid this problem, alternative control methods have been proposed,
including the use of selective insecticides such as soybean oil and abamectin (both naturally derived) and imidacloprid (neonicotinoid) together with parasitoid biological control.

Soybean (Glycine max L.) oil, which is extracted from seeds, is safe and not present in the ecosystem (Lee et al. 2005). Similar to other oil-based products, soybean oil acts by causing insect death through contact via disruption of gas exchange or epidermal cell membrane function as well as spiracle obstruction, which causes asphyxia (Pless et al. 1995). Abamectin, the mixture of avermectin B₁₉ and B₂₁₉, is produced during fermentation by the soil actinomycete Streptomyces avermitilis (ex Burg et al.) Kim and Goodfellows (Lasota and Dybas 1991). This biopesticide persistently stimulates membrane function as well as spiracle obstruction, which causes mortality (LC₅₀) and sublethal (adult emergence, sex ratio, and adult longevity) effects on target and non-target organisms (Morales et al. 2013). Therefore, this feeding behavior appears to enhance the potential of T. triozae as a biological control agent. However, few studies have addressed the toxicity of insecticides toward T. triozae, and most existing studies only focused on acute toxicity (Luna-Cruz et al. 2011, 2015; Liu et al. 2012; Martinez et al. 2015). Nonetheless, as the importance of other adverse effects has been highlighted by modern ecotoxicology (Desneux et al. 2007), a complete analysis of their impacts requires that the potential sublethal effects caused by insecticides on this parasitoid must be considered in addition to the lethal effects.

The objective of this study was to evaluate the lethal (direct mortality) and sublethal (adult emergence, sex ratio, and adult longevity) effects of three insecticides on immature stages of T. triozae: two synthetic products that affect the host's haemolymph (host feeding), which also contributes to overall mortality (Morales et al. 2013). Therefore, this feeding behavior appears to enhance the potential of T. triozae as a biological control agent.

Materials and Methods

Insects

The insects used in the assays derive from permanent colonies of B. cockerelli and T. triozae maintained at the Entomology Laboratory of the Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Mexico. The rearing of B. cockerelli was completed within a greenhouse (16–30°C, 60% relative humidity [RH], and an 14–10 [L:D] h photoperiod) using tomato plants (Ríos Grande) for feeding of nymphs and adults and as an oviposition substrate (Martinez et al. 2015). Rearing of the parasitoid T. triozae was performed in the laboratory (25°C, 56% RH, and a 12:12 [L:D] h photoperiod) by using tomato plants infested with a mixture of third-, fourth-, and fifth-instar nymphs of B. cockerelli suitable for the development of immature stages and by providing adults with honey ad libitum (Morales et al. 2013, Martinez et al. 2015).

Chemicals

The commercial products AbaKrone (1.8% abamectin, emulsion concentrate), ImidaKrone (30.2% imidacloprid, suspension concentrate), and EPA 90 (90% refined soybean seed oil, emulsion concentrate) were used in this study. EPA 90, registered in Mexico under the code RSCO-INAC-0103F-301-009-090, is identical to that registered under code 031605 by the U.S. Environmental Protection Agency. All of these insecticides were obtained from Biokrone S. A de C.V., Celaya, Guanajuato, Mexico.

Lethal and Sublethal Effects

The lethal effects of abamectin, imidacloprid, and soybean oil on the eggs, larvae, and pupae of T. triozae were examined separately using parasitized B. cockerelli nymphs containing parasitoids in these different development stages. The methodology of Martinez et al. (2015) was followed to obtain T. triozae eggs, larvae, and pupae of the same age. In brief, tomato leaflets bearing 15 fourth-instar B. cockerelli individuals (54 h after ecdysis) were offered to 13-d-old parasitoid females with previous copulation for 6 h; this age was chosen because it is at which maximum oviposition activity occurs in this species. The leaflets were carefully examined under a stereoscopic microscope; nymphs that had not been parasitized were removed using a small brush.

Parasitized nymphs of B. cockerelli were incubated under the laboratory conditions described above for T. triozae rearing to obtain larval and pupal stages of T. triozae (2 and 5 d after parasitization, respectively); parasitized nymphs containing the egg stage of the parasitoid were used the same day the parasitization occurred. Leaflets containing B. cockerelli nymphs parasitized with eggs (<10 h old), larvae (<12 h old) or pupae (<12 h old) of T. triozae were dipped for 5 s into one of three concentrations of abamectin (9, 4.5, or 0.03 mg [Al]/liter), imidacloprid (260, 130, or 3 mg [Al]/liter), or soybean oil (1,620, 810, or 443 mg [Al]/liter). Immersion is a common practice in studies assessing the efficacy of pesticides against pests or their non-target effects against natural enemies (Hassan 1977). The concentrations bioassayed correspond to the minimum field-registered concentration (100% MiFRC), half that concentration (50% MiFRC), and the median lethal concentration (LC₅₀) for B. cockerelli fourth-instar nymphs, respectively (Bujanos et al. 2005). To enhance the wetting of the leaflet, the surfactant Tween 20 was used at 0.01% (w/v). Control leaflets were dipped in distilled water plus surfactant at 0.01%. Six replicates were performed for each concentration of each insecticide as well as each immature developmental stage bioassayed. Ten parasitized fourth-instar nymphs of B. cockerelli containing each developmental stage of T. triozae were considered to be one replicate.

The treated tomato leaflets were dried at room temperature in a well-ventilated area (2 h after treatment). Next, each leaflet was individually transferred to an insect-free frame box (14 × 14 × 14 cm) and maintained under the laboratory conditions described above. The mortality of both the eggs and larvae of the parasitoid was recorded every 24 h post-treatment until the formation of pupae. Eggs and larvae that were flabby or dried were considered dead. Mortality of pupae derived from treated eggs and larvae, as well as that of treated pupae, was recorded after emergence of adult parasitoids.

The numbers of parasitoid adults that emerged from treated nymphs were recorded at 14, 11, and 7 d after insecticide application of the egg, larval, and pupal stages of the parasitoid, respectively. After emergence, the sex ratio of adults was calculated as the percentage of females [females/(females + males) × 100]. Adult longevity was also recorded. For this, the adults that emerged from
each treated immature stage as well as in each insecticide treatment at different concentrations were placed in a cylindrical plastic tube (5.5 cm in diameter and 10.5 cm in height) that was open at both ends. The top of the cylinder was covered with a fine mesh screen to permit air circulation and to prevent the insects from escaping. In both the control and treatment groups, the parasitoids were fed small drops of pure honey deposited on the inner cylinder walls and were given water ad libitum. The adults were checked every 24 h until they died.

Data Analysis
The experiment comprised a 4 × 3 factorial design with treatment (four levels: control, abamectin, imidacloprid, and soybean oil) and treated stage (three levels: egg, larva, and pupa) as the main factors. The data were subjected to analysis of variance (ANOVA) after checking for normality and homoscedasticity. Analyses were performed using the fixed-effects model, with the least significant difference (LSD) multiple range test (P < 0.05) to separate means (SAS/STAT version 9.3; SAS Institute, Cary, NC).

Results
Lethal Effects
Treated Eggs
No significant differences in the mortality of eggs were observed among the soybean oil concentrations, but all of the groups treated with soybean oil showed significant differences from the controls (F = 10.49; df = 7, 33; P = 0.001) (Table 1). For imidacloprid, no significant differences were observed between the highest concentration (1% mortality) and the controls (0%). Regarding abamectin, mortality (6%) was only assessed at the lowest concentration (0.03 mg [AI]/liter) because this compound caused 100% mortality of host nymphs at the other two concentrations evaluated.

Treated Pupae
No mortality was observed for T. triozae larvae obtained from eggs treated with soybean oil, but significant effects (F = 10.51; df = 7, 33; P = 0.001) were observed for imidacloprid (mortality ranging from 4 to 25%) and the lowest concentration of abamectin (39%) (Table 1). No significant differences in mortality were found between parasitoid pupae derived from treated eggs and those derived from control eggs (F = 0.54; df = 7, 33; P = 0.790).

Treated Larvae
The insecticides soybean oil, imidacloprid, and abamectin significantly affected (F = 4.24; df = 7, 40; P = 0.001) the larval stage of T. triozae (Table 1). Mortality due to soybean oil and imidacloprid was very similar, ranging from 11 to 19% for both insecticides, except for the lowest concentration of soybean oil, for which 6% mortality was observed. No significant differences in mortality were found between larvae treated with the two lowest concentrations of abamectin (≤4.5%) and the control (2%). Conversely, the highest concentration of abamectin caused 100% mortality in B. cockerelli nymphs. The mortality of T. triozae pupae obtained from treated larvae was only higher than the control at the three concentrations of imidacloprid (~10%) (F = 3.23; df = 8, 40; P = 0.006) (Table 1).

Treated Larvae
The insecticides soybean oil, imidacloprid, and abamectin significantly affected (F = 4.24; df = 7, 40; P = 0.001) the larval stage of T. triozae (Table 1). Mortality due to soybean oil and imidacloprid was very similar, ranging from 11 to 19% for both insecticides, except for the lowest concentration of soybean oil, for which 6% mortality was observed. No significant differences in mortality were found between larvae treated with the two lowest concentrations of abamectin (≤4.5%) and the control (2%). Conversely, the highest concentration of abamectin caused 100% mortality in B. cockerelli nymphs. The mortality of T. triozae pupae obtained from treated larvae was only higher than the control at the three concentrations of imidacloprid (~10%) (F = 3.23; df = 8, 40; P = 0.006) (Table 1).

Table 1. Mortality (mean ± SE) of eggs and larvae of Tamarixia triozae when parasitized fourth-instar Bactericera cockerelli were treated with three insecticides by dipping

| Treatment     | Conc (mg [AI]/liter) | Mortality (%) |
|---------------|----------------------|---------------|
|               | Treated eggs         | Larvae from treated eggs | Pupae from treated eggs |
| Control       | 0                    | 0 ± 0A        | 0 ± 0A |
| Soybean oil   | 443                  | 26 ± 2C       | 0 ± 0A |
|               | 810                  | 36 ± 2C       | 0 ± 0A |
|               | 1,620                | 61 ± 1C       | 2 ± 2A |
| Imidacloprid  | 3                    | 12 ± 1B       | 3 ± 1AB|
|               | 130                  | 6 ± 1B        | 7 ± 1B |
|               | 260                  | 1 ± 1A        | 25 ± 1C|
| Abamectin     | 0.03                 | 6 ± 1B        | 39 ± 1C|
|               | 4.5                  | ND            | ND     |
|               | 9                    | ND            | ND     |
| Treated larvae|                      | —             | —      |
| Control       | 0                    | 2 ± 1A        | 0 ± 0A |
| Soybean oil   | 443                  | 6 ± 1AB       | 2 ± 1A |
|               | 810                  | 19 ± 1C       | 2 ± 1A |
|               | 1,620                | 15 ± 1B       | 2 ± 1A |
| Imidacloprid  | 3                    | 11 ± 1B       | 10 ± 1B|
|               | 130                  | 19 ± 1BC      | 8 ± 1B |
|               | 260                  | 16 ± 1BC      | 9 ± 1B |
| Abamectin     | 0.03                 | 4 ± 1AB       | 3 ± 2A |
|               | 4.5                  | 0 ± 0A        | 1 ± 1A |
|               | 9                    | ND            | ND     |

Means within columns that are followed by the same letter are not significantly different (P > 0.01). ND, Not determined because 100% mortality was observed for parasitized Bactericera cockerelli nymphs.
of the lowest concentration of imidacloprid, the mortality caused by the other two concentrations of this compound and all of the abamectin doses differed significantly \((F = 27.28; \text{df} = 19, 100; P = 0.006)\) from the controls (0%).

**Sublethal Effects**

Emergence of *T. triozae* adults developed from treated eggs and larvae declined significantly \((F = 10.35; \text{df} = 26, 123; P = 0.001)\) when parasitized fourth-instar *B. cockerelli* were treated with the three compounds (Table 2). When the parasitoid eggs were treated with soybean oil and imidacloprid, adult emergence declined 1.6- to 3.6-fold and 1.4- to 1.6-fold compared with the control, respectively, whereas adult emergence decreased 11-fold when eggs were treated with abamectin at the lowest concentration (Table 2). Emergence of adults obtained from larvae treated with soybean oil and abamectin was only significantly reduced at 810 and 4.5 mg (AI/liter relative to the controls (1.6- and 10-fold, respectively) (Table 2). In the case of imidacloprid, adult emergence was significantly reduced (by 1.6-fold) at 130 and 260 mg (AI/liter) (Table 2). The emergence of adult *T. triozae* obtained from treated pupae was very similar for soybean oil and imidacloprid (~90–100%), and no significant differences were observed compared with the controls (Table 2). With regard to abamectin, the lowest two concentrations significantly reduced adult emergence (2- and 5-fold) compared with the controls.

The sex ratio of *T. triozae* was significantly affected when insects were treated as larvae \((F = 2.1; \text{df} = 7, 340; P = 0.043)\) with concentrations of 810 and 1620 mg (AI/liter soybean oil (66 and 68% females, respectively) or when treated as pupae \((F = 4.03; \text{df} = 9, 47; P = 0.001)\) with 3 and 260 mg (AI/liter) imidacloprid (60 and 58% females, respectively). The effect was a reduction in the female proportion compared to the control insects (83–84% females) (Table 3). When parasitoids were treated as eggs, the percentage of females emerging was not significantly altered \((F = 1.98; \text{df} = 7, 230; P = 0.058)\) by any concentration of either soybean oil or imidacloprid or by the lower concentration of abamectin (range 61–91%) compared to the control (78%).

The longevity of adults obtained from treated immature stages of *T. triozae* depended on the concentration of insecticide used (Table 4). Regardless of the parasitoid stage, the longevity of males was generally between 22 and 37 d. Significant differences \((F = 3.66; \text{df} = 19, 199; P = 0.001)\) were only observed in males emerging from larvae treated with 0.03 mg (AI/liter) abamectin and those derived from pupae treated with 260 mg (AI/liter) imidacloprid and 0.03 and 9 mg (AI/liter) abamectin, for which the longevity was between 17 and 21 d.

### Table 2. Emergence of *Tamarixia triozae* adults (mean ± SE) from treated (by dipping) egg, larval, and pupal stages when parasitizing fourth-instar *Bactericera cockerelli*

| Treatment     | Concn (mg [AI]/liter) | Egg emergence (%) | Larva emergence (%) | Pupa emergence (%) |
|---------------|------------------------|-------------------|--------------------|-------------------|
| Control       | 0                      | 100 ± 0Aa         | 96 ± 2Aa           | 100 ± 0Aa         |
| Soybean oil   | 443                    | 63 ± 3Ba          | 80 ± 4ABa          | 100 ± 0Aa         |
|               | 810                    | 50 ± 12Ba         | 61 ± 8Ba           | 100 ± 0Ab         |
|               | 1,620                  | 28 ± 2Ca          | 68 ± 5ABb          | 100 ± 0Ab         |
| Imidacloprid  | 3                      | 71 ± 4Ba          | 78 ± 2ACa          | 100 ± 0Aa         |
|               | 130                    | 65 ± 7Ba          | 59 ± 5BCa          | 92 ± 4Aa          |
|               | 260                    | 62 ± 6Ba          | 60 ± 6BCa          | 88 ± 3Aa          |
| Abamectin     | 0.03                   | 9 ± 6Da           | 71 ± 3ABb          | 83 ± 8Ab          |
|               | 4.5                    | ND                | 10 ± 0Da           | 33 ± 8Bb          |
|               | 9                      | ND                | 28 ± 9B            |                   |

Means within columns (capital letters) and within rows (lowercase letters) that are followed by the same letter are not significantly different \((P > 0.01)\). ND, Not determined because 100% mortality was observed for parasitized *Bactericera cockerelli* nymphs.

### Table 3. Adult sex ratios (means ± SE) of *Tamarixia triozae* derived from egg, larval, and pupal stages when parasitized fourth-instar *Bactericera cockerelli* were treated with three insecticides by dipping

| Treatment     | Concn (mg [AI]/liter) | Eggs (% female) | Larvae (% female) | Pupa (% female) |
|---------------|------------------------|-----------------|-------------------|-----------------|
| Control       | 0                      | 78 ± 5a         | 84 ± 5b           | 83 ± 5b         |
| Soybean oil   | 443                    | 78 ± 9a         | 75 ± 6ab          | 87 ± 4b         |
|               | 810                    | 93 ± 10a        | 68 ± 8a           | 85 ± 5b         |
|               | 1,620                  | 61 ± 10a        | 66 ± 7a           | 83 ± 6b         |
| Imidacloprid  | 3                      | 79 ± 6a         | 87 ± 5b           | 60 ± 6a         |
|               | 130                    | 77 ± 6a         | 74 ± 7ab          | 82 ± 5b         |
|               | 260                    | 97 ± 6a         | 73 ± 7ab          | 58 ± 7a         |
| Abamectin     | 0.03                   | 91 ± 1a         | 91 ± 4b           | 74 ± 6ab        |
|               | 4.5                    | ND               | ND                | 84 ± 6b         |
|               | 9                      | ND               | ND                | 94 ± 6b         |

Means within columns (lowercase letters) followed by the same letter do not differ significantly \((P > 0.01)\). ND, Not determined because 100% mortality was observed for parasitized *Bactericera cockerelli* nymphs.
Table 4. Longevity (days ± SE) of Tamarixia triozae adults derived from egg, larval, and pupal stages when parasitized fourth-instar Bactericera cockerelli were treated with three insecticides by dipping

| Treatment   | Conc (mg [Al]/l) | Longevity of Tamarixia triozae adults developing from different immature stages (d) |
|-------------|------------------|----------------------------------------------------------------------------------------------------------------------------------|
|             |                  | Egg                                                                 | Larva                                                                 | Pupa                                                                 |
|             |                  | Male          | Female         | Male          | Female         | Male          | Female         |
| Control     | 0                | 27 ± 1ABa     | 23 ± 1ABa      | 33 ± 1Bb      | 42 ± 1Ba      | 32 ± 1Bb      | 34 ± 1Bb      |
| Soybean oil | 443              | 15 ± 1Aa      | 25 ± 1AA       | 27 ± 1ABA     | 32 ± 1ABA     | 25 ± 1ABA     | 22 ± 1AA      |
|             | 810              | ND            | 43 ± 1CE       | 25 ± 1ABA     | 40 ± 1BB      | 24 ± 1ABa     | 30 ± 1BDa     |
|             | 1,620            | 25 ± 1ABa     | 30 ± 1BCa      | 27 ± 1Aa      | 31 ± 1BAa     | 25 ± 1ABA     | 23 ± 1ACDa    |
| Imidacloprid| 3                | 32 ± 1Ba      | 33 ± 1CEDa     | 22 ± 1Aa      | 33 ± 1AAa     | 25 ± 1BAa     | 29 ± 1BCa     |
|             | 130              | 27 ± 1ABA     | 35 ± 1CEDa     | 24 ± 1ABA     | 28 ± 1AAa     | 22 ± 1BAa     | 36 ± 1Ba      |
|             | 260              | ND            | 44 ± 1E        | 26 ± 1ABA     | 30 ± 1ABa     | 18 ± 1Aa      | 32 ± 1Bb      |
| Abamectin   | 0.03             | 30 ± 1ABa     | 16 ± 1AAa      | 20 ± 1Aa      | 26 ± 1Aa      | 21 ± 1Aa      | 20 ± 1Aa      |
|             | 4.5              | —             | —              | —             | —             | —             | —             |
|             | 9                | —             | —              | —             | —             | —             | 17 ± 1Aa      | 27 ± 1ABCa    |

Means within columns (capital letters) and within rows (lowercase letters) for each stage (egg, larva and pupa) that are followed by the same letter do not differ significantly (P > 0.01). ND, Not determined because males died between 5 and 7 d after emergence, before the start of the bioassay. —, Death of Bactericera cockerelli nymphs containing eggs or larvae of the parasitoid Tamarixia triozae.

Female longevity varied between 20 and 40 d for all insecticides, their concentrations, and immature parasitoid life stages tested (Table 4), with no significant difference from the control (23, 42, and 34 d for females obtained from eggs, larvae, and pupae, respectively). However, there were three exceptions: 1) females obtained from eggs treated with 810 mg (Al)/liter soybean oil at 3, 130, or 260 mg (Al)/liter imidacloprid lived significantly longer than the controls; 2) the controls lived significantly longer than females obtained from larvae treated with 130 mg (Al)/liter imidacloprid or 0.03 mg (Al)/liter abamectin; and 3) the controls lived significantly longer than females obtained from pupae treated with 443 or 1,620 mg (Al)/liter soybean oil or 0.03 or 4.5 mg (Al)/liter abamectin.

For each developmental stage of the parasitoid T. triozae, no significant differences in longevity were observed between males and females across all treatments, with the exception of adults obtained from larvae treated with 810 mg (Al)/liter soybean oil at 1, 3, or 130 mg (Al)/liter imidacloprid, which survived significantly longer than the controls (F = 1.91; df = 15, 57; P = 0.041) and those obtained from pupae treated with 260 mg (Al)/liter abamectin (F = 2.24; df = 19, 72; P = 0.007); in these cases, the longevity of males was significantly shorter than that of females.

There is no information available in the literature about the lethal effects of soybean oil, imidacloprid, and abamectin on the egg and larval stages of the ectoparasitoid T. triozae, though many studies have been conducted on the lethal effects of the above-mentioned compounds on the immature stages of endoparasitoids. In this regard, imidacloprid and abamectin were found to cause 32 and 51% mortality, respectively, to the preimaginal 'egg-larval' stage of the parasitoid Trichogramma pretiosum (Riley; Hymenoptera: Trichogrammatidae), with both compounds causing 30% mortality to the prepupal stage (Carvalho et al. 2003). For the larvae of the ectoparasitoid Tamarixia radiata (Waterston; Hymenoptera: Eulophidae), abamectin at a concentration of 40 mg (Al)/liter resulted in high (93%) mortality when parasitized fourth-instar Diaphorina citri (Kuwayama; Hemiptera: Psyllidae) nymphs were treated by spraying (Santos et al. 2015). In contrast, low mortality of T. triozae larvae at the two highest concentrations of this compound (130 and 260 mg [Al]/liter) (between 16 and 19%) was observed in our study, which can be taken as evidence of a higher tolerance to this compound or differences in mortality based on the mode of application.

Similar to our study, a high concentration (2 liter/ha) of a mineral oil (Biolide) caused 13% mortality to larvae of the ectoparasitoid Aphytis melinus (DeBach; Hymenoptera: Aphelinidae) when parasitizing nymphs of the oleander scale Aspidiotus nerii (Bouche; Hemiptera: Diaspididae) (Biondi et al. 2015). In contrast, mineral oil (Sunspray) resulted in 98, 84, and 55% mortality to eggs, young larvae, and old larvae, respectively, of Encarsia pergandiella (Howard; Hymenoptera: Aphelinidae) when parasitizing nymphs of Bemisia argentifolii (Bellows & Perring; Hemiptera: Aleyrodidae) (Stansly and Liu 1997).

In the present study, the pupal stage of T. triozae was the least susceptible to both soybean oil and imidacloprid. This was likely because T. triozae is an ectoparasitoid; thus, and at the end of its development, it fixes the remains of the dead psyllid nymph to the plant surface to pupate under it as a protective barrier. However, some studies have reported that other mineral oils and imidacloprid cause moderate to high mortality to both endo- and ectoparasitoids at this developmental stage. For example, 99% mortality to Enc. pergandiella pupae was found when the nymphs of its host, B. argentifolii, were treated with a low concentration of Sunspray (2.9 mg (Al)/liter of abamectin) with a high concentration of abamectin (260 mg (Al)/liter).
effect of soybean oil, imidacloprid, and abamectin toward aphid parasites and paralysis.

It is important to note that in our study, parasitized B. cockerelli nymphs were completely drenched with the insecticide solution; consequently, they were exposed to a much higher amount of the insecticides than under normal practice. Therefore, the acute toxicity caused by soybean oil, imidacloprid, and abamectin toward T. triozae may be associated with the following three factors. First, insecticides can cause immediate host death once they contact the host nymph body (Mahdavi et al. 2011, Sohrabi et al. 2013), as observed in the present study with abamectin, which caused total mortality of B. cockerelli nymphs at two (9 and 4.5 mg [AI]/liter) of the three concentrations evaluated. Second, after treatment, pesticides are deposited between the underside of the nymph and the leaf surface, which is the preferred location of the immature stages of T. triozae. Consequently, parasitoid mortality may occur through direct contact with the pesticide. Third, pesticides can cause indirect juvenile parasitoid mortality if the parasitoids develop outside of the host and feed on contaminated individuals (Kumar et al. 2008, Mahdavi et al. 2011). Accordingly, T. triozae eggs and larvae coated with soybean oil solution exhibited clear symptoms of dehydration, and larvae treated with imidacloprid and abamectin displayed tremors and paralysis.

T. triozae adult emergence was significantly reduced by the three insecticides tested, depending on the timing of the application, but no general pattern was detected. The decline in adult emergence caused by soybean oil, imidacloprid, and abamectin appeared to be dose dependent in some cases across all three developmental stages, and this effect was most evident in adults obtained from treated eggs and larvae. Variable results in terms of adult emergence have also been observed in other species of endo- and ectoparasitoids when treated at different immature stages with imidacloprid, abamectin, and oil-based compounds. For example, in En. inaron (Sohrabi et al. 2012), both En. formosa and Er. eremicus (Sugiyama et al. 2011), and Er. mundus (Sohrabi et al. 2013), adult emergences were 15, 45, and 48%, respectively, when treated at the pupal stage with these compounds. In contrast, 100% emergence was observed when T. radiata was treated at the pupal stage (Belotti et al. 2015).

In addition, when pupae were treated with abamectin, adult emergence was 35–41% (Ferrari 2009) for T. radiata and 58% for T. triozae (Luna-Cruz et al. 2011), whereas emergence was ≤5% in Eretmocerus warrae Naumann & Schmidt (Hymenoptera: Aphelinidae) and Trichogramma chilonis (Sugiyama et al. 2011) and Er. mundus (Sohrabi et al. 2013), adult emergences were 15, 45, and 48%, respectively, when treated at the pupal stage with these compounds. In contrast, 100% emergence was observed when T. radiata was treated at the pupal stage (Belotti et al. 2015).

When interpreting data on emergence rates from insecticide-treated hosts, it is also important to consider the fitness of the parasitoids that emerge (Smilanick et al. 1996). In our study, the sex ratio and adult longevity of T. triozae were examined to assess the sublethal effects of the tested insecticides. It has been reported that in T. triozae, the sex ratio normally favors females (range 86–88%) (Morales et al. 2013, Rojas et al. 2015). When we treated T. triozae eggs, larvae, and pupae with soybean oil, imidacloprid, and abamectin, the proportion of females was not affected, except when the parasitoid was treated as larvae with the 50 or 100% MiFRC of soybean oil or when treated as pupae with the LC_{50} or 100% MiFRC of imidacloprid; in these cases, there were significantly more males than females. Given that no such effect was observed with the 50% MiFRC of imidacloprid, the biological significance of this finding remains unclear. Additionally, when the parasitoid A. melinus was treated as larvae with mineral oil (Biondi et al. 2015) or when Bracon nigricans (Szépligeti; Hymenoptera: Braconidae) was treated as pupae with emamectin benzoate (compound of the same family as abamectin, avermectins) (Biondi et al. 2013), the sex ratio favored females (64 and 81%, respectively, for each parasitoid), as observed in En. inaron (Sohrabi et al. 2012), T. pretiosum (Carvalho et al. 2003), Habrobracon hebetor (Say; Hymenoptera: Braconidae) (Rafiee et al. 2012), and A. ervi (Zuazúa et al. 2003) when treated with imidacloprid as larvae or pupae.

In contrast to our results, the number of males was higher in B. nigricans when pupae were treated with abamectin (Biondi et al. 2013), and the number of A. colemani males also increased following treatment at the pupal stage with an insecticide based on saponified olive and neem oils (Tremblay et al. 2008). Although the mechanisms by which these effects might be exerted are poorly understood, we hypothesize that they may be associated with several factors: the number and age of spermatozoa present in spermathecae; the death and reabsorption of spermatozoa; the number of copulations; and differential mortality of sexes in the larval stage (Holloway et al. 1999, Damiens et al. 2003).

Most studies about the effects of chemical insecticides on the longevity of parasitoid adults obtained from treated immature stages have addressed the longevity of females, and relatively little has been published regarding that of males (Rafiee et al. 2012; Sohrabi et al. 2012, 2013). We observed that the longevity of T. triozae adults was affected according to the concentration and type of insecticide applied and by the immature stage treated. However, no inter-sex differences were observed, with the exception of 810 mg (AI/liter) soybean oil and 260 mg (AI/liter) abamectin, for which females obtained from both treated larvae and pupae lived 14–15 d longer than males. In another study, the longevity of adults of the endoparasitoid Bracon brevicornis (Wesmael; Hymenoptera: Braconidae) obtained from parasitized Spodoptera littoralis (Boisdouval; Lepidoptera: Noctuidae) larvae was 1 and 2 d longer for females than males when treated at egg and larval stages, respectively, with the neonicotinoid compound thiamethoxam or thiacloprid at 1.5 mg (AI/liter) (Tabozada et al. 2015).

Regardless, Kumar et al. (2008), reported that females of Er. warrae obtained from eggs, larvae, and pupae treated with 2 ml (AI/liter) abamectin lived 1 d longer than males (3 vs. 2 d). The only case we found in the literature showing a strongly unbalanced effect on longevity was that of the parasitoid B. nigricans, whereby female adults derived from pupae that developed with the host, Tuta absoluta (Meyrick; Lepidoptera: Gelechiidae), treated with 150 and 75 ml (AI)/liter emamectin benzoate and abamectin, respectively, lived ~20 d longer when males (Biondi et al. 2013). It is important to note that the effects on...
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