SHORT COMMUNICATION

Nontarget Effects of Aerial Mosquito Adulticiding With Water-Based Unsynergized Pyrethroids on Honey Bees and Other Beneficial Insects in an Agricultural Ecosystem of North Greece

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ABSTRACT We assessed the nontarget effects of ultra-low-volume (ULV) aerial adulticiding with two new water-based, unsynergized pyrethroid formulations, Aqua-K-Othrine (FFAST antievaporant technology, 2% deltamethrin) and Pesguard S102 (10% d-phenothrin). A helicopter with GPS navigation technology was used. One application rate was tested per formulation that corresponded to 1.00 g (AI)/ha of deltamethrin and 7.50 g (AI)/ha of d-phenothrin. Three beneficial nontarget organisms were used: honey bees (domesticated hives), family Apidae (*Apis mellifera* L.); mealybug destroyers, family Coccinellidae (*Cryptolaemus montrouzieri* Mulsant); and green lacewings, family Chrysopidae (*Chrysoperla carnea* (Stephens)). No significant nontarget mortalities were observed. No bees exhibited signs of sublethal exposure to insecticides. Beehives exposed to the insecticidal applications remained healthy and productive, performed as well as the control hives and increased in weight (25–30%), in adult bee population (14–18%), and in brood population (15–19%).

KEY WORDS aerial adulticiding, unsynergized pyrethroid, nontarget, bee

Aerial ultra-low-volume (ULV) applications of mosquito adulticides involve applying low rates of insecticides from aircraft (10–100 ml/ha, depending on the formulation; Latham and Barber 2007) in the form of an aerosol spray, using an efficient droplet size range ($Dv_{0.5}$ <60 μm and $Dv_{0.9}$ <100 μm, as per label recommendations), to target flying adult mosquitoes. To maximize the spray efficacy, the applications occur during crepuscular or nocturnal hours when some major nuisance and pathogen-transmitting mosquitoes are active, while nontarget beneficial organisms, such as bees and butterflies, are not (Connelly and Carlson 2009).

Aqua-K-Othrine and Pesguard S102 are two water-based, unsynergized formulations of deltamethrin and d-phenothrin, respectively, available for mosquito adulticiding in Europe. Both deltamethin (type II pyrethroid) and d-phenothrin (type I pyrethroid) are toxic to beneficial insects such as honey bees (LC_{50}s 0.05 and 0.067 μg per bee for deltamethrin and d-phenothrin, respectively, National Pesticide Information Center [NPIC] 2010). No studies exist on acute effects of aerial ULV applications of unsynergized deltamethrin and d-phenotrhin to beneficial terrestrial insects.

Little information is available on the effects of vector control applications on bees (Zhong et al. 2003, 2004; Boyce et al. 2007), and most literature involves the immediate exposure of caged bees to the insecticidal cloud during crepuscular hours (Colburn and Langford 1970, Womeldorf et al. 1974, Caron 1979, Pankiw and Jay 1992, Boyce et al. 2007). These field bioassays do not take into account the biology of the bees, which return to their hives during the night (Seeley 1996). Zhong et al. (2004) investigated the effects of aerial ULV applications of the organophosphate Naled on beehives; however, the effects (acute and sublethal) of aerial pyrethroid ULV application on beehives have not been investigated.

ULV aerial adulticiding applications using two water-based pyrethroids caused high mosquito mortality during experimental trials conducted in the rice field ecosystem of northern Greece (Chaskopoulou et al. 2011). The majority of the treatment areas received accurate and uniform coverage by the spray cloud as evidenced by the high droplet densities ($Dv_{0.5}$ 35–40 μm) and uniform caged mosquito mortalities observed within the treatment sites. In this manuscript we report: 1) the acute or immediate toxic effects of the aforementioned aerial applications of the two mosquito adulticides on terrestrial beneficial insects, in-

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14. ABSTRACT
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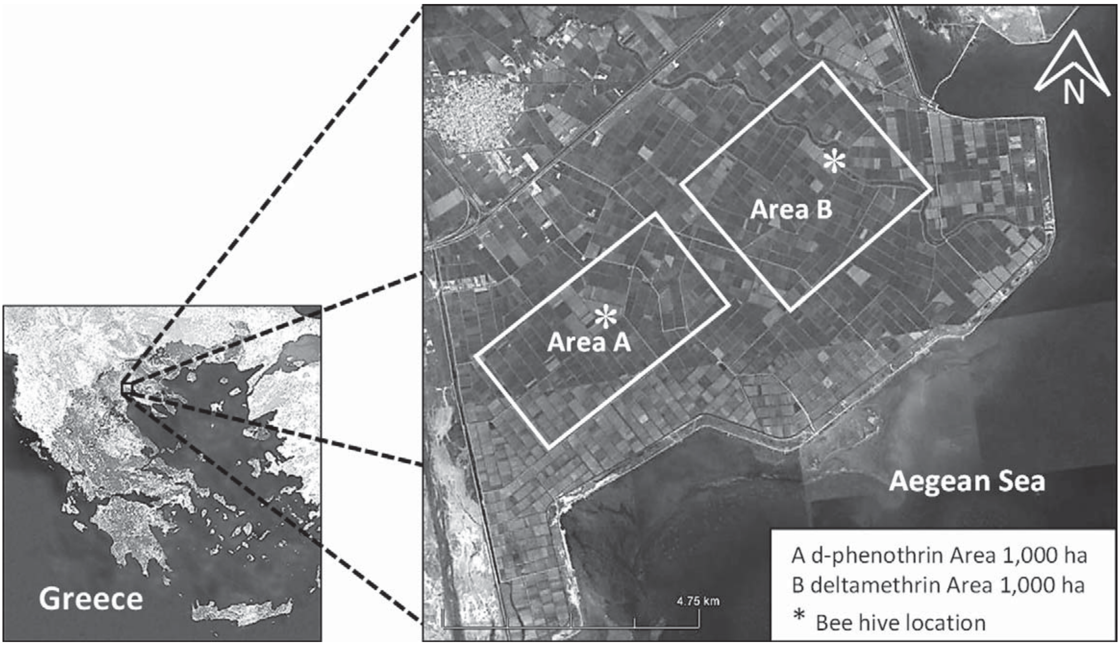

Fig. 1. A satellite image of the two treatment areas (2009 experimental season) located in the agricultural area of West Thessaloniki, Greece. The location of the beehives are indicated by (*).

cluding honey bees, and 2) the sublethal effects of the products on the performance of beehives.

Materials and Methods

Insecticides and Application Technologies. Two commercially available ULV insecticides registered in Europe for ground mosquito adulticiding were used for the aerial treatments: Aqua-K-Othrine (2% wt:wt deltamethrin; Bayer Environmental Science, Lyon, France) and Pesguard S102 (10% wt:wt d-phenothrin; Sumitomo Chemical Co. Ltd., Osaka, Japan). Over the 2 yr, five spray trials were conducted with each product based on recommendations of the World Health Organization (WHO) for ULV applications of insecticides for mosquito adulticiding (WHO 1997) using label rates registered in other countries (1.0 g/ha of active ingredient [AI]) for Aqua-K-Othrine and 7.5 g/ha [AI] for Pesguard S102).

Insecticide applications were conducted using a turbine helicopter (500C model; McDonnell Douglas Helicopters, Mesa, AZ). Information on application technologies (nozzle type and navigational systems) and flight parameters (altitude, weather conditions, swath widths, and offsets) are provided elsewhere (Chaskopoulou et al. 2011).

Experimental Sites. During 2008, two experimental sites were chosen: a treatment site (Area A), where all insecticide treatments were conducted, and a control site, where no treatments took place. During 2009, beehives were added in the experiments and because the bees had to be exposed separately to each insecticide, an additional treatment site was included (Area B) that would allow for each insecticide to be applied at a separate site (Fig. 1). The treatment sites were open agricultural areas ≈1,000 ha in size (A = ≈4 by 2.5 km and B = ≈3.5 by 3.21 km). The control sites assigned for both years were similar to the treatment sites and located ≈3–5 km from the treatment areas.

Nontarget Insects. Three terrestrial beneficial insects were used: green lacewing larvae *Chrysoperla carnae* Stephens (Neuroptera: Chrysopidae), adult mealybug destroyers *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), and domesticated honey bees *Apis mellifera* L. (Hymenoptera: Apidae).

Chrysoperla larvae were imported from a European biological control company (Koppert, Berkel en Rodenrijs, The Netherlands). The larvae were received as first and second instars in bottles and were maintained in plastic rearing containers in an incubator at 15°C. *Chrysoperla* larvae were fed *Ephestia kuhniella* Zeller (Lepidoptera: Pyralidae) eggs and aphids. The use of aphids as a food source significantly reduced the degree of cannibalism in the rearing containers. For the experiments, third- and fourth-instar larvae were used. In previous research, *Chrysoperla* larvae that survived insecticide exposure were able to pupate and successfully emerge as adults (Nasreen et al. 2005). Approximately 24 h before the spraying trial, the larvae were moved from the incubator to the laboratory at 23°C to acclimatize them for field exposure. Adult *C. montrouzieri* were purchased from a Greek biological control company (BioInsecta, Thessaloniki, Greece). The adults were received the day of the experiment and were used immediately.

The bees were exposed to the treatments within their hives. Fifteen hives (Langstroth, 51 by 41 by 24 cm) were obtained from Aristotle University at Thessaloniki and were delivered to the experimental sites (five hives in Area A, five hives in Area B, and five hives in the control area) 2 wk before the experiments to allow for bee acclimatization. The hives were placed in the center of the treatment sites to ensure exposure to the majority of the insecticidal cloud (Fig. 1). Previous studies have shown that hives located well within an insecticide treated area sustain more losses than hives situated elsewhere (Anderson and Atkins 1968). The hives were left in the experimental sites for the entire summer season when the trials were conducted (≈2.5 mo). Upon delivery, each hive contained a minimum of four brood frames and ≈12,000 adult bees and was provided with syrup that the bees could access in case food sources within their new environment were insufficient.

Assessment Bioassay. Sampling stations: 8–14 sampling stations were deployed at preassigned locations within each treatment site, and 5 sampling stations were placed in the control area. All sampling stations were deployed and handled in a similar manner. Each sampling station contained holding devices for each nontarget insect species except for the bees, which were in their hives.

Beneficial insects: Every sampling station had one open 237-ml container (American Plastics, Chattanooga, TN) containing 10 *C. montrouzieri,* and two containers containing 5 *C. carnae* each to prevent cannibalism resulting from overcrowding. To prevent insects from escaping, a thin film of Vaseline was applied along the upper edge of the containers. The containers with the insects were placed in the field ≈15 min before the spray application commenced and remained there until ≈30 min after the spraying was completed to ensure the entire insecticidal cloud passed through the intended area. Approximately 60 min postspraying, all containers were returned to the laboratory.

The beehives were monitored for presence of dead bees the morning before, 12 h postspraying, and at weekly intervals (for 10 wk). All of the beehives were fitted with entrance traps to collect the dead bees. In addition to acute bee mortality, adult bee and brood populations, and weight of each hive, were recorded weekly. The presence of shiny bees (bees with fallen setae—indicative of sublethal insecticide exposure) was recorded by careful observation of each frame for the presence of unusual looking adult bees.

Statistical Analysis. All statistical analyses were performed with the SAS software package (SAS Institute Inc., Cary, NC). Five replicates were analyzed per insecticide. One-way analysis of variance (ANOVA) was performed to determine the effect of the insecticide treatment on percentage *Chrysoperla* and *Cryptolaemus* insect mortality. Data were arcsine-square-root-transformed before ANOVA. Means were separated using Student–Newman–Keuls ($P = 0.05$; [SAS Institute Inc. 2003]).

A paired *t*-test was performed to determine any insecticide effect on the development of the beehives ($P = 0.05$; [SAS Institute Inc. 2003]). Adult bee and brood populations, and the weight of the beehives before (beginning of the summer) and after treatments (end of the summer), were compared for all treatment and control areas.

Table 1. Mortality of nontarget organisms exposed to the aerial adulticide treatments with Pesguard and Aqua-K-Othrine, in Thessaloniki, Greece

Treatment	Application rate (ml/ha)	Replicates (*N*)	% mortality (mean ± SEM)[a]	
			Cryptolaemus adults	*Chrysoperla* larvae
AKO	50.0	5	1.9 ± 1.0[a]	2.5 ± 1.1a
PESG	75.0	5	3.3 ± 1.2[a]	1.7 ± 0.8a
Control	0.0	5	1.0 ± 1.0[a]	3.3 ± 1.6a

AKO, Aqua-K-Othrine; PESG, Pesguard S102.

[a] Means within a column followed by the same letter are not significantly different ($P = 0.05$, Student–Newman–Keuls [SAS Institute Inc. 2003]).

Results

Insect Acute Mortality. All nontarget insect species had very low mortalities when exposed to the products tested (Table 1). Mean *C. carnae* mortality was 2.5 and 1.7% and mean *C. montrouzieri* mortality was 1.9 and 3.3% when exposed to Aqua-K-Othrine and Pesguard, respectively. Control mortalities remained low and were not significantly different than the treatment mortalities. There was no unusual mortality of adult bees and the average daily mortality never exceeded 10 dead bees per hive (Fig. 2). No shiny bees were observed in any hive.

Beehive Performance. The beehives in the two treatment areas performed similarly to the beehives in the untreated area, and increased in weight (25–30%), adult bees (14–18%), and brood population size (15–19%). There was no significant increase in adult bee populations at all three areas (Table 2); however, there was a significant increase in brood for the treatment Area B and the control area. There was a significant increase in the total weight of the beehives in all three areas. The increase in weight is attributed not only to the increase of brood and adult population but also to honey production.

Discussion

The aerial ULV trials resulted in high droplet densities within our treatment plots (400–4,000 drops per square centimeter on slides) and high mosquito control levels in both sentinel-caged mosquitoes and wild populations (Chaskopoulou et al. 2011). However, we observed no significant mortality of nontarget insects exposed to the treatments. When mosquito adulticides are delivered in appropriate droplet size, similar to the one used in the research presented here, there is low ground deposition of pesticides and no measurable negative effect to nontarget species (Dukes et al. 2004). Crop protection through the release of biological control agents, such as *Chrysoperla* and *Cryptolaemus,* is an important practice in Greece (Tzanakakis 1980). Our study provides evidence that aerial ULV

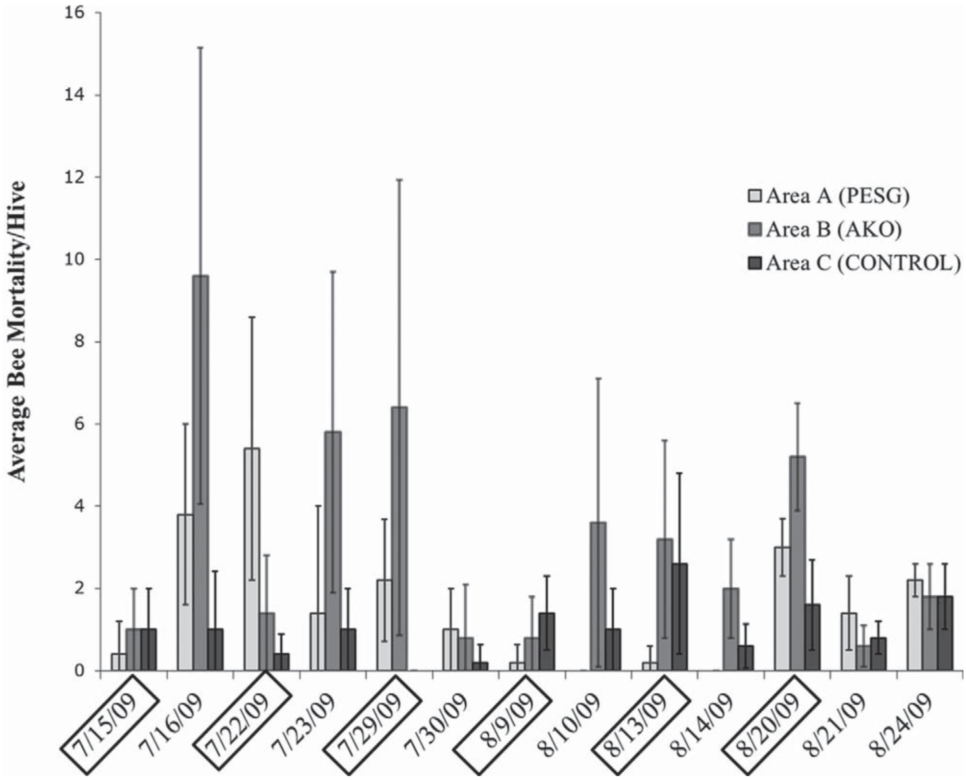

Fig. 2. Average daily bee mortality (±SD) sampled the morning before (dates in boxes) and the morning after (dates without boxes) each spray application.

mosquito adulticiding, when applied properly, has no significant effects on these biological control agents.

Despite honey bees being highly susceptible to both products tested very low bee mortalities, not exceeding 20 bees per hive daily, were observed in experimental beehives. Normal daily bee mortality varies from 20 to 25 (Delabie et al. 1985), to 30–80 (Gary and Mussen 1984), and ≤100 bees per hive (Johansen 1977). Pesticides cause higher losses to bees when applied during day (Byrne and Waller 1990), but late evening spraying when the majority of bees are not foraging and are sheltered in beehives (Seeley 1996) results in minimal exposure of bees to the insecticides. Insecticide residues on water, or any nectar and pollen source, could contaminate the hive and stress or kill the bees. For example, sublethal doses of parathion slow the bee foraging flight and affect the perception of time in foraging bees (Desneux et al. 2007) and beehive productivity, whereas several chemicals reduce bee egg hatch or brood production (Erickson and Erickson 1983). In our study, the beehives in the treated areas performed well and increased adult bee population, brood, and weight, suggesting that insecticide deposits had no biologically significant effect on bees foraging within the treatment areas.

With the increasing prevalence of mosquito-borne pathogenic diseases in the European community, such as the recent West Nile Virus epidemic in North Greece with 261 human cases and 34 deaths (Hellenic Centre for Disease Prevention and Control [HCDC] 2010), pyrethroids, such as d-phenothrin and deltamethrin, will be more widely used to control mosqui-

Table 2. Performance of beehives (mean ± SEM) before and after exposure to aerial adulticiding treatments with Pesguard and Aqua-K-Othrine, in Thessaloniki, Greece

Treatment	Replicates (N)	Adult population (frames per hive)[a]		Brood population (frames per hive)		Weight (kg per hive)	
		Before	After	Before	After	Before	After
AKO	5	8.8 ± 2.7a	10.4 ± 0.5a	6.0 ± 1.2a	6.9 ± 1.1a	23.4 ± 2.5a	30.4 ± 4.7b
PESG	5	8.4 ± 1.5a	9.6 ± 1.1a	6.2 ± 0.8a	7.4 ± 0.9b	24.0 ± 3.0a	30.0 ± 2.9b
Control	5	10.0 ± 0.0a	10.4 ± 0.5a	6.2 ± 0.8a	7.6 ± 1.1b	22.2 ± 3.4a	26.6 ± 3.2b

AKO, Aqua-K-Othrine; PESG, Pesguard S102.

[a] Means before and after the treatments within a row followed by the same letter are not significantly different ($P = 0.05$, Student's t-test [SAS Institute Inc. 2003]).

toes. It is increasingly important to use appropriate methods that maximize efficacy against mosquitoes while minimizing environmental impact. Aerial ULV adulticiding with the products evaluated in this study can be done with no acute and sublethal effects on beneficial insects. This adult mosquito control methodology is environmentally sound, even over sensitive environments containing honey bees, as long as late evening applications using proper spray technologies (nozzles and navigational systems) are used.

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