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Doses and Residues of Acephate Baits Used to Eradicate Undesirable Honey Bees: A Hazard Assessment

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Africanized honey bees (neotropical hybrids of *Apis mellifera scutellata* L.) have generated much scientific, regulatory and public interest because of their potential negative impacts on agriculture and public health (McDowell 1984; Taylor 1985; Rinderer 1986). Eliminating Africanized colonies from localized areas is a goal of federal and state regulatory agencies charged with protecting the U.S. beekeeping industry (USDA 1987; Maxwell 1989); destroying nuisance colonies also will be important to minimize stinging incidences in the general public. Until recently, no technology was available for efficiently suppressing localized populations of undesirable honey bees. To fill this void, a baiting system was developed which can be used to destroy remotely located colonies from relatively long distances (Danka et al. 1989; Williams et al. 1989). The process begins as bees are attracted to sucrose-honey syrup baits during periods of low nectar availability. Foragers passively become trained to collect untreated syrup, and then later deliver lethal doses of acephate (*O,S*-dimethyl acetylphosphoramidothioate) to their nest after untreated syrup is replaced with syrup containing acephate.

The discovery of the expansion of the Africanized bee population into south Texas in October 1990 is expected to intensify calls for bee population control. Prior to any implementation of acephate baiting, it is necessary to assess the environmental hazards posed by the technique. This report addresses this issue by summarizing information on acephate doses delivered to experimental colonies under field conditions typifying anticipated regulatory situations. In addition, residues of acephate and its metabolite methamidophos in treated colonies were measured to evaluate their potential environmental contamination.

MATERIALS AND METHODS

Foragers from individual active colonies of ca. 20,000-50,000 bees were trained to isolated feeders containing 50% sucrose solution (volume:volume)

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plus 10% honey by volume. Appropriate treatment distances (100-1000 m) were obtained by moving feeders with foraging bees away from hives during several days of training. Alternatively, about half of the colonies were treated following tests of effectiveness of baits in luring bees (Danka et al., 1989; in press). In these cases, foragers from colonies were baited to feeders at distances of ca. 90-700 m from hives prior to treatment.

For treatments in all tests, feeders having untreated training syrup were replaced with feeders having 500 ppm (mg/L) acephate (Orthene® 75 S) in syrup during active bee visitation (100 to 300 bees foraging simultaneously). Foraging persisted for ca. 20-30 min before bees became intoxicated; feeders were removed promptly when visitation ceased. Dosage data were obtained from differences in syrup weights taken before and after treatment. Colony health was monitored until it was determined that the colonies were permanently queenless (and thus were destroyed) or that they had survived the treatment.

Residues of acephate and its metabolite methamidophos were measured in samples of dead adult bees and honey-beeswax matrix taken from five colonies. These colonies had collected 35 ± 13 mg (mean ± standard deviation) of acephate (in 69 ± 27 mL of treated syrup) from baits 500 m from their nests. Dead bees that accumulated inside each hive and in 56- X 47-cm screen trays at hive entrances were held in an empty hive chamber directly beneath the colony; 25- to 30-g composite samples of mixed-age bees were taken for analyses. For honey and beeswax analyses, five 3- × 3-cm sections of comb, each taken from the same sides of the same five alternating combs, were composited as a colony sample. The sample sections contained wax, sealed honey and unsealed honey from one side of the comb midrib. Samples were stored at -10 $^{\circ}$ C until analyses, which usually occurred within 2 wk. Each colony was sampled once before treatment and then 11 times spanning 10 wk after treatment.

Insecticide residues were extracted using a modified version of Luke's (1983) general method; details are given here because of the difficulty in extracting acephate and methamidophos, especially from honey. A 20 g sample of bees or honey-wax matrix was homogenized for 10 min with 130 mL of acetone and 70 mL of water at medium high speed in a Waring blender. After vacuum filtering through sharkskin filter paper (prerinsed three times with acetone and three times with ethanol), an 80 mL aliquot was removed to a 500 mL separatory funnel and 100 mL of dichloromethane plus 100 mL of petroleum ether were added. This mix was shaken vigorously for 1 min and then allowed to separate. The lower aqueous layer was drained into a graduated cylinder. The upper organic layer was drained through anhydrous sodium sulfate (prerinsed three times with acetone and three times with ethanol) into a sample beaker. After the aqueous layer was returned to the

separatory funnel, 7 g of sodium chloride was added and dissolved by shaking for 30 sec. The aqueous layer was rinsed with 100 mL of dichloromethane, shaken for 1 min and allowed to separate. The lower layer, now organic, was drained through sodium sulfate and combined in the sample beaker. The aqueous layer was rinsed with an additional 100 mL of dichloromethane. Finally, 50 mL of dichloromethane was used to rinse the sodium sulfate. The solvents were evaporated to 2 mL in a 40 ^{o} C water bath. To remove dichloromethane residues, 10 ml of acetone was added and the sample was reconcentrated to 2 mL; this was done three times. The samples were transferred to centrifuge tubes with acetone, and concentrated to a 5 g/mL equivalent using a stream of nitrogen. Equivalent concentrations were calculated as: g sample × (80 mL aliquot / 200 mL extracting solvent) × (1 / mL final volume) = g sample / mL final extract.

Residues in the extracts were quantified on two different columns in a gas chromatograph equipped with dual nitrogen-phosphorous detectors. One column was a DB-5 fused silica wide bore capillary (30 m × 0.53 mm). The other column was a glass column (1.2 m × 2 mm) packed with 2% Stabilized DEGS on 80-100 mesh Chromosorb W HP (no glass wool at inlet). Operating temperatures were 190 ^{o} C isothermal for the column oven, 200 ^{o} C at the injection port and 250 ^{o} C at the detector. Gas flow rates were helium carrier at 25 mL/min, hydrogen at 3.5 mL/min and air at 50 mL/min. Quantification was made by comparing peak heights of samples to those of analytical standards (acephate, 99.7%; methamidophos, 98.6%). The final residue level for each colony at each sampling time was calculated as the average of residues found using the two different columns. Fortification levels and recoveries ranged from 51 ppm acephate (84% recovery) and 48.8 ppm methamidophos (89% recovery) in the honey-wax matrix to 0.1 ppm acephate (80% recovery) and 0.09 ppm methamidophos (80% recovery) in dead bees.

RESULTS AND DISCUSSION

Several aspects of these field trials suggest that eradicating honey bees by acephate baiting presents minimal environmental hazard. Most importantly, the baiting system was effective against honey bee colonies when only small amounts of acephate were delivered during treatment. Overall, target colonies collected an average of 30 mg (± 11 mg; n=30) of acephate and 34 of 35 colonies died after treatment at distances of 88-1000 m (i.e., distances expected under field conditions). This effective dose is very low relative to recommended rates of acephate use against a variety of pests. For example, 30 mg is 1/50-1/150 the amount of acephate (2 to 6 g [one teaspoon to one tablespoon] of Orthene® 75 S) labeled for use against individual colonies of red imported fire ants, *Solenopsis invicta* Buren. Furthermore, Orthene® is labeled for use against up to 13 fire ant colonies per acre annually, or up to 78 g Orthene® (58.5 g acephate)/acre/year. Such an amount of acephate

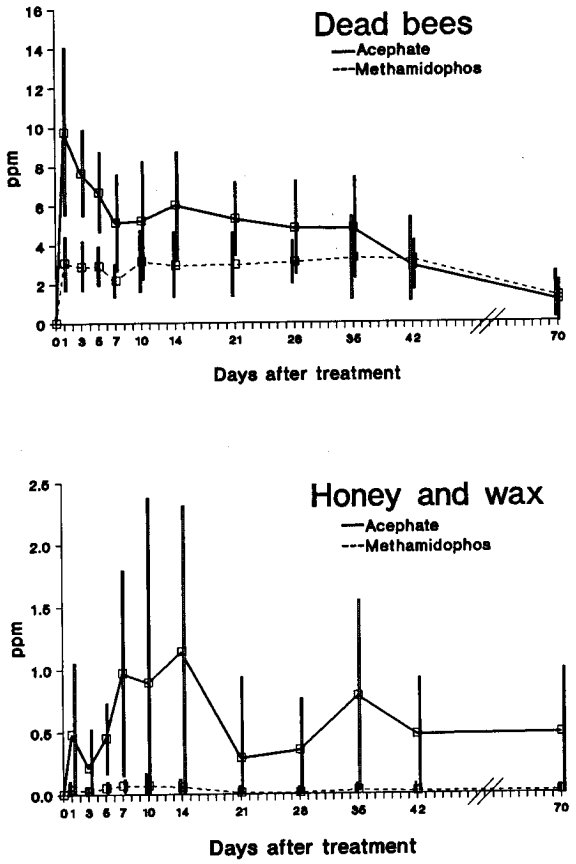

Figure 1. Residues (mean ± standard deviation) of acephate and methamidophos recovered from dead bees and honey-wax matrices of five colonies treated with 500 ppm acephate at 500 m.

potentially could be used to eradicate nearly 2000 honey bee colonies, which is the number of feral colonies typically found across several hundred square miles in temperate areas of the United States (Taber 1979; Visscher and Seeley 1989; Morse et al. 1990). The effectiveness of the low doses used in the baiting system probably results from the direct delivery of acephate in syrup and the trophallactic dispensation of the material among nestmates, including the queen.

Residues of the small amounts of insecticide collected were themselves relatively low within treated colonies. Acephate residues were higher than those of methamidophos, and residues of both compounds were higher in dead bees than in the honey-wax matrix (Fig. 1). Acephate levels in dead bees peaked near 10 ppm (μg/g) one day after treatment and declined to near 1.0 ppm 10 wk later. Methamidophos levels in dead bees remained

near 3.0 ppm for the first six wk after treatment, then declined to ca. 1.3 ppm at 10 wk. The relative stability of residues in dead bees, especially during later sampling sessions, probably reflects a concentration of residues as sampled bees dried.

Both insecticides apparently were transferred at low levels to stored honey (Fig. 1). No residues of these very hydrophilous compounds were expected from beeswax. Residues remained low ($\leq$1.1 ppm acephate; $<$0.1 ppm methamidophos) and showed no clear decay throughout sampling. This stability indicates no conversion of acephate to methamidophos within the honey; dependence on specific amidases is known for this conversion (Magee 1982). The low residues suggest little danger would be posed if animals, including humans, were to eat honey from treated nests. Acephate has low mammalian toxicity (acute oral LD_{50} [rat] of ca. 900 mg/kg). Methamidophos is much more toxic (acute oral LD_{50} [rat] of ca. 20 mg/kg), but was detected at very minimal levels in the honey-wax matrix. Acephate and methamidophos in combination have tolerances of up to 10 ppm established for fresh foodstuffs (lettuce and celery) (Chemical Regulation Reporter 1989).

The baiting technique is quite specific in treating only honey bees. Unlike systems wherein toxic baits are broadcast (e.g., for fire ants), bees collect treated syrup from a contained point source during a brief presentation of treated syrup. The efficient recruitment system of honey bees results in high activity of bees at the feeder, and this excludes almost all feeding by other species. In addition, the treatment is carefully supervised, and the feeder is removed as soon as bee visitation declines. During treatments of more than 100 honey bee colonies, very few individuals of other insect species and no mammals or birds have fed on acephate baits. Honey bees are much more sensitive to acute acephate poisoning than are *Bombus terrestris* L. and *Episyrphus balteatus* De Geer (Drescher and Geusen-Pfister, in press).

In conclusion, only small doses of acephate are required to successfully eradicate target honey bee colonies by baiting, minimal residues remain in destroyed nests, and nontarget organisms rarely are exposed during treatment. These factors lend support to the acephate baiting system as a relatively safe means of helping manage problems arising from Africanized honey bees.

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