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Ovicidal and Larvicidal Effects of Certain Herbicides on Honey Bees[1,2,3]

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ABSTRACT

The herbicides 2,4-D, 2,4,5-T, silvex, 2,4-DB, dicamba, 2,3,6-TBA, picloram, chloramben, dalapon, and EPTC were fed in 60% sucrose-water solution to *Apis mellifera* L. colonies to determine their effects on brood production. Picloram, 2,3,6-TBA, and dicamba had no adverse effects on brood development at a concentration of 1000 parts per million active ingredient by weight (ppmw). Chloramben and dalapon caused reduction in brood development and 2,4-D, 2,4,5-T, silvex, 2,4-DB, and EPTC severely reduced or eliminated brood production at this concentration. Both ester and salt formulations of the phenoxy herbicides prevented brood development. The phenoxy herbicides, when fed at concentrations of 10 ppmw, caused no adverse effect on brood development but reduced amount of brood when fed at concentrations of 100 ppmw. The eggs did not hatch in colonies fed the higher levels of phenoxy herbicides. The adverse effects of phenoxy herbicides on brood development were temporary and once the herbicide was removed brood development was resumed.

In previous studies (Moffett et al. 1972, Morton et al. 1972) we found that adult honey bees, *Apis mellifera* L., were relatively unaffected by substituted phenoxy, substituted benzoic, and substituted picolinic acid herbicides, either through exposure by feeding or spraying. Herbicides containing arsenic and paraquat were toxic when fed to or sprayed on adult honey bees. Most of the adverse effect of herbicides on honey bees has been attributed to loss of food when plants on which honey bees forage for nectar and pollen are killed by the herbicides (Hocking 1959). Many compounds have been evaluated for their effects on reproduction of undesirable insects but few have been evaluated for their effects on honey bees. Palmer-Jones (1964) reported distress and a 20% mortality in field honey bees following aerial application of a mixture of sodium salt of 2,4-D and superphosphate, but he observed no adverse effect on brood or hive activity 12 and 16 days after treatment. Taber and Bořkovec (1969) were able to prevent development of diploid worker honey bees by treating semen in vitro with the chemosterilant tepa, and artificially inseminating queens with treated semen. They found no appreciable effect of tepa on viability of unfertilized eggs.

This study was initiated to determine if those herbicides which were relatively nontoxic to adult worker honey bees would influence nonsexual reproduction in the colony (egg laying by the queen, nurse bees caring for larvae, mortality of larvae and pupae, and emergence of adults from the pupal stage).

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[3] Company and trade names are given for identification purposes only and do not constitute endorsement by the U.S. Department of Agriculture or Arizona Agricultural Experiment Station.
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Materials and Methods

To obtain the equivalent of a newly established swarm of bees, we placed 3 lb of honey bees and a laying, fertile queen on each frames of beeswax foundation. As soon as the bees were placed in the hive they were fed herbicides. Unless otherwise noted, all herbicides were fed at a concentration of 1000 parts per million active ingredient by weight (ppmw) in 60% sucrose-water solution from a 1-qt jar placed directly above the frame containing brood. The mixture of herbicide and syrup was replenished twice a week. The jar was protected by an empty super. A maintenance diet consisting of 1 part pollen, 2 parts soyflour, and 6 parts sucrose with enough water added to make a moist cake was also placed on top of the frames. Each hive was placed in a 12×12×9-ft Saran®-mesh cage. There were 9 cages in which we placed hives and we usually conducted a trial with 2 herbicide treatments and nontreated check. Each test was replicated 3 times. Fresh water was constantly available in a 5-qt plastic pail. The herbicides were fed for at least 21 days.

We measured brood production by: (1) recording the number of frames having eggs, larvae, pupae, or emerging adults; (2) delineating 100 cells and recording the number of empty cells, larvae, pupae, and emerging adults; and (3) measuring the area of sealed and unsealed brood. Brood production in the honey bee colony is a dynamic process and is dependent upon many factors: genetics of the colony; nutrition of the colony; age, condition, and fecundity of the queen; climatic conditions; and season of the year. Because of the variability in reproduction, we are expressing all data concerning brood development in colonies that were fed herbicides as percent of the check colonies. Brood production was estimated at frequent intervals and always on the 1st, 3rd, 8th, 15th, and 21st days after the 1st eggs were observed in the colony.

The common[6] and trade names of commercially formulated herbicides fed to honey bees are: triethylamine salt of 2,4,5-T (Veon® 245); dimethylamine salt of 2,4-D (DMA-4®); propylene glycol butyl ether esters of silvex (Kuron®); dimethylamine salt of dicamba (Banvel® D4S); dimethylamine salts of 2,3,6-TBA and other chlorinated benzoic acids (Benzac® 1281); potassium salt of picloram (Tordan® 22K); sodium salt of dalapon (Dowpon®); EPTC (Eptam® 6E). The following herbicides of at least technical purity or better were fed to honey bee colonies: acid of 2,4,5-T[7]; triethylamine salt of 2,4,5-T[7]; sodium salt of 2,4,5-T[7]; butoxyethanol ester of 2,4-D; sodium salt of 2,4-DB; sodium salt of chloramben.

Results and Discussion

Studies with 2,4,5-T

The triethylamine salt of 2,4,5-T fed at 1000 ppmw prevented brood development in the honey bee colony (Table 1). The 1st eggs laid by the queen appeared to be normal in size and abundance; however, after the colonies had been fed 2,4,5-T for 15 days the eggs were less numerous. The 1st larvae to appear were normal in appearance and abundance; however, they were only 40 and 20% as abundant on the 15th and 21st days, respectively, as in the check colonies. Although 10% of these larvae pupated, all died before the 21st day; and no adult worker bees emerged from the cells in colonies fed 2,4,5-T.

We further studied the effect of 2,4,5-T on honey bee brood by feeding the triethylamine salt of 2,4,5-T at 10, 100, and 1000 ppmw, and the sodium salt of 2,4,5-T and the acid of 2,4,5-T at 1000 ppmw, for 21 days. Colonies fed the triethylamine salt of 2,4,5-T at 10 ppmw produced as much brood as the check colonies (Table 2). Brood production was inhibited in colonies fed the triethylamine salt of 2,4,5-T at 100 ppmw. Both triethylamine and sodium salts of 2,4,5-T inhibited brood production. The triethylamine salt of 2,4,5-T was slightly more active than the sodium salt. Approximately 1% of the eggs laid in the colonies fed sodium salt hatched, pupated, and developed into adult worker honey bees. The acid of 2,4,5-T was somewhat less inhibitory than the salts; however, the lower inhibition probably was due to low solubility of 2,4,5-T in 60% sucrose-water solution. We homogenized the acid of 2,4,5-T in 60% sucrose with a blender, but some of it settled out after 48 hr in the feeding jars. Since the sodium and triethylamine salts and the acid of 2,4,5-T inhibited brood production, it is evident that formulation of 2,4,5-T is not critical and the 2,4,5-T molecule causes the inhibition. We removed the sodium and triethylamine salts of 2,4,5-T from colonies on the 21st day, but kept the colonies under observation for an additional 25 days. All colonies were observed on the 25th day after herbicides were removed and all stages of brood were found in each of the colonies.

Studies with Other Phenoxy Herbicides

All phenoxy herbicides inhibited brood development at the high levels of treatment (Table 2). The dimethylamine salt of 2,4-D was especially effective in preventing brood development and in reducing egg laying at 100 and 500 ppmw and eliminated egg

Table 1.—Development of honey bee brood expressed as percent of check as influenced by triethylamine salt of 2,4,5-T at 1000 ppmw in 60% sucrose solution.

Stage of brood development	% indicated day after 1st eggs laid				
	0	3	8	15	21
Eggs	100	100	100	50	50
Larvae	—	100	50	40	20
Pupae	—	0	10	10	0
Adults	—	—	—	—	0

[6] Common names of herbicides are those approved by the Weed Science Society of America, 1971, as listed in Weed Science 19(5), back cover.

[7] Concentration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin less than 0.5 ppmw.

Table 2.—Development of honey bee brood expressed as percent of check as influenced by herbicides.

Herbicide	Concn in 60% sucrose-water solution (ppmw)	Amount of brood 21 days after 1st eggs were laid			
		Eggs	Unsealed	Sealed	Emerging adults
2,4,5-T, triethylamine salt	10[b]	100	100	100	100
2,4,5-T, triethylamine salt	100[b]	70	50	25	1
2,4,5-T, triethylamine salt	1000[b]	50	0	0	0
2,4,5-T, sodium salt	1000[b]	50	0	5	1
2,4,5-T, acid	1000[b]	75	0	10	1
2,4-D, dimethylamine salt	100[a]	50	10	5	1
2,4-D, dimethylamine salt	500[a]	40	0	0	0
2,4-D, dimethylamine salt	1000[a]	0	0	0	0
2,4-D, butoxyethanol ester	1000[b]	50	25	5	2
Silvex, propylene glycol butyl ether ester	1000[a]	90	75	50	25
2,4-DB, sodium salt	1000[b]	90	40	20	20
Dicamba, dimethylamine salt	1000[a]	100	100	100	100
2,3,6-TBA, dimethylamine salt	1000[a]	100	100	100	100
Chloramben, sodium salt	1000[b]	100	90	75	75
Picloram, potassim salt	10[a]	105	105	105	105
Picloram, potassim salt	1000[a]	120	120	120	120
Dalapon, sodium salt	1000[a]	100	75	50	50
EPTC, emulsifiable concentrate	1000[a]	60	50	20	0

[a] Herbicide in commercial formulation.
[b] Herbicide of at least technical purity.

laying at 1000 ppmw. The butoxyethanol ester of 2,4-D was also inhibitory but less so than the amine salt. Although it may actually be less inhibitory than the salt when mixed with 60% sucrose, it formed an emulsion which tended to break after 24 hr and some of the ester of 2,4-D floated on the surface of the 60% sucrose-water solution. The lower inhibition by ester of 2,4-D may have been due to a lower concentration of 2,4-D at the feeding orifices of the jars rather than effect of formulation.

Silvex caused the least inhibition of brood production of the phenoxy herbicides tested at 1000 ppmw. The emulsion formed by the ester of silvex and 60% sucrose was as stable as that formed by the ester of 2,4-D; thus, silvex appears to be less inhibitory to honey bee brood production than 2,4-D.

Sodium salt of 2,4-DB inhibited brood production less than either the ester or salt formulations of 2,4-D but slightly more than the ester of silvex.

On the 12th feeding day we transferred some eggs from colonies fed 2,4-D at 100 and 500 ppmw, to petri dishes which contained filter paper impregnated with beeswax. The technique described by Taber (1961) was used to transfer the eggs. From each of the 9 colonies, 120 eggs were transferred, placing 30 eggs in each dish. The dishes containing eggs were incubated at 34°C and observed twice daily for 72 hr after transfer. Eggs from colonies fed no herbicide gave 85% hatch, and 65% of eggs hatched from colonies fed 2,4-D at 100 ppmw. No eggs hatched from colonies fed at 500 ppmw.

We wanted to determine if 2,4-D inhibited other stages of brood development as well as hatching. We continued to feed dimethylamine salt of 2,4-D and butoxyethanol ester of 2,4-D to colonies beyond 21 days. The queen and approximately 50 g of workers were confined in a cage within the colony on empty, drawn comb. After 2 days of confinement, each frame on which the queen had been confined and had laid eggs was transferred to another queenless colony. At least 2 colonies subjected to each herbicide treatment originated and received frames on which eggs were laid.

Eggs on frames transferred from colonies consuming no herbicide to other colonies consuming no herbicide hatched and developed all stages of brood. Eggs transferred from colonies consuming no herbicide to colonies consuming dimethylamine salt of 2,4-D hatched; however, all larvae died within 3 days. Eggs transferred from colonies consuming no herbicide to colonies consuming butoxyethanol ester of 2,4-D hatched but 95% of the larvae died within 3 days. A few brood cells were capped, however, no adults emereged and all cells examined contained dead larvae or pupae. Eggs did not hatch when they were transferred from colonies consuming dimethylamine salt of 2,4-D or butoxyethanol ester of 2,4-D to colonies consuming no herbicide.

We also transferred queens from colonies which had been consuming dimethylamine salt of 2,4-D for 21 days to colonies which consumed no herbicide. Five days after transfer we observed many eggs in colonies receiving the queens but no larvae. Eight days after transfer, in addition to numerous eggs, these colonies also contained small larvae, and 11 days after transfer we observed eggs, small and large larvae, and capped brood cells. These colonies and queens were kept under observation for several months. Healthy brood was found at each observation and no abnormal condition was detected.

Studies with Benzoic and Related Herbicides

Dicamba and 2,3,6-TBA did not inhibit brood production (Table 2). Chloramben did not reduce egg laying but caused a slight reduction in numbers of larvae, pupae, and adult worker bees.

Studies with Other Herbicides

We found no inhibition of brood development when potassium salt of picloram was fed to honey bee colonies; in fact, there was a nonsignificant increase in the amount of brood produced in colonies receiving picloram when compared with the check colonies (Table 2).

Colonies consuming dalapon produced less brood than check colonies (Table 2). Egg laying was not inhibited by dalapon and only a slight reduction in numbers of larvae was noted. The larvae seemed to pupate normally; however, 21 days after 1st eggs were laid, only 50% as much sealed brood was present in the colonies fed dalapon as in check colonies. Dalapon did not appear to inhibit egg hatching, as was the case with the phenoxy herbicides. Gouck and LaBrecque (1964) found that methyl-2,3-dichloropropionate inhibited pupation of *Musca domestica* L. and high rates killed females during the preoviposition period. The chemical similarity of this compound to dalapon is evident. Dalapon was not particularly toxic to adult worker bees in this study or to newly emerged adult workers (Morton et al. 1972).

EPTC caused considerable reduction in brood production. Eggs were always present in colonies consuming EPTC, but 15 and 21 days after 1st eggs were laid they were less abundant than in check colonies (Table 2). The numbers of larvae in the colonies fed EPTC were extremely variable. No live larvae were seen in colonies 8 days after 1st eggs were laid; however, larvae were observed in colonies 15 and 21 days after 1st eggs were laid. Pupae were 20% as abundant in colonies fed EPTC as in check colonies, but we did not observe any emerging adults. Colonies consuming EPTC produced less comb wax than check colonies. This undoubtedly influenced brood production indirectly as the space for brood was smaller than in check colonies. EPTC has been shown to reduce the amount of epicuticular wax in peas, *Pisum sativum* L. (Still et al. 1970), and apparently interferes with lipid metabolism in honey bees. EPTC is used primarily as a soil-incorporated herbicide and the probability of it injuring honey bees when used as recommended is remote.

Our study shows that a number of herbicides, notably the phenoxy acids, prevent reproduction in the honey bee colony and may fall in Bořkovec's (1966) definition of a chemosterilant. Eggs from colonies consuming 2,4-D did not hatch and larvae died when transferred into colonies consuming this herbicide. The effects of phenoxy herbicides on reproduction are temporary. Once the herbicide was removed from the honey bee colony, brood development resumed.

The phenoxy herbicides are applied primarily as sprays and could be carried back to the colony if applied to vegetation on which honey bees forage or could be deposited in their source of water. The amount of 2,4-D that could be carried would be far below that required to have an effect as shown in this study. We were unable to detect 2,4-D or 2,4,5-T in honey sacs of honey bees or in colonies sprayed by airplanes with 2,4-D or 2,4,5-T (Moffett and Morton 1971). These herbicides usually kill flowers rapidly, and flowers which are not killed usually wilt and nectar secretion is inhibited or stopped.[8] Such damage to plants is probably the main reason herbicides are unlikely to be carried from plants to honey bee colonies. Herbicides are more likely to injure honey bee colonies by depriving them of their source of food in the treated area by killing plants on which they forage for nectar and pollen than by reducing brood production.

[8] C. C. King. 1961. Effects of herbicides on honey bees and nectar secretion. Doctoral diss. Ohio State University, Univ. Microfilms, Inc., Ann Arbor, Mich. 177 p.

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