Technical Bulletin No. 114 October, 1947

University of Arizona
COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION

HONEYBEE LOSSES AS RELATED TO CROP DUSTING WITH ARSENICALS

By
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PUBLISHED BY
University of Arizona
TUCSON. ARIZONA

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TABLE OF CONTENTS

ILLUSTRATIONS

HONEYBEE LOSSES AS RELATED TO CROP DUSTING WITH ARSENICALS[1]

By S. E. McGregor, A. B. Caster, and Marvin H. Frost, Jr.

INTRODUCTION

Honeybee losses in Arizona resulting from the dusting of crops with arsenicals have been heavy in recent years. The Arizona State Beekeepers' Association reports that in 1942 over 7,500 colonies were estimated to have been killed in this way. Losses in 1943 were not determined, but appear to have been less severe. In 1944 approximately 2,000 colonies were killed or seriously damaged in areas near insecticide-dusting crops. In 1945 more than 7,000 colonies were killed and as many more were seriously damaged.

Such losses have forced many beekeepers to go out of business or to move to other locations; others have taken the loss and restocked their apiaries, only to suffer similar losses in following years. Similar reports issue from many other states, wherever crops are treated with arsenical insecticides.

The relation of this wholesale destruction of bees to reduction in yield of those crops requiring the services of such insects as pollinators is unknown, but the damage is doubtless much greater than is generally realized. Considerable evidence exists that a decline in the production of legume seed in various areas is directly connected with the decline in honeybee populations.

The purpose of this study was to obtain factual information to show the relationship between airplane application of the various types of arsenical dusts in common use and honeybee losses.

The lack of definite information pertinent to the high mortality of bee colonies in areas where arsenical dusts have been applied has made impossible a practical solution of this important economic problem. Beekeepers, farmers, and commercial airplane crop dusters recognized the desirability of obtaining such information. In the interest of these groups a co-operative study was instituted by the Bureau of Entomology and Plant Quarantine and the Arizona Agricultural Experiment Station to obtain this much needed information.

[1]A co-operative research project between the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Agricultural Research Administration, and the Arizona Agricultural Experiment Station: S. E. McGregor and Marvin H. Frost, Jr., for the Division of Bee Culture of the Bureau of Entomology and Plant Quarantine, and A. B, Caster for the Arizona Agricultural Experiment Station.

Appreciation is expressed to the cotton farmers, beekeepers, and airplane crop dusters for their co-operation in this work; to the Botany Department of the University of Arizona for the identification of plant specimens; and to C. E. Burnside, of the Bureau of Entomology and Plant Quarantine, for assistance in the analytical work.

LITERATURE REVIEW

A survey of the literature reveals a history of increasing losses of bees from poisoning with insecticides. The first record of the use of arsenic as an insecticide in the United States was in 1868, when Paris green was used to control potato insects (1, 43). In 1881 Thompson (48) observed that bees were killed after Paris green had been sprayed on a pear tree in full bloom. In 1892 the insecticidal value of lead arsenate was discovered, and it soon became the leading stomach poison for insects (22). The use of this insecticide in the control of the codling moth on fruit trees resulted in losses of bees and other pollinating insects. Decrease in bee losses, better control of the codling moth, and less injury to fruit resulted when the growers applied the insecticide before or after the bloom periods. Some bee losses have continued, however, in connection with the spraying of fruit trees with arsenicals, apparently because of the contamination of blossoms still attractive to bees, or of cover-crop blossoms beneath the sprayed trees, and because at times the bees collect the liquid spray.

In 1921 Melander (36) expressed hope that the recently developed calcium arsenate might be less destructive than the lead arsenate and that dry dusting might be better than spraying. Bishop (6) reported in 1923 that in his area of Nova Scotia dry dusting caused more damage to bees than did spraying, his explanation being that the small, dry particles drifted farther than the spray mist; he also believed that the bees carried the dust to their hives in the pollen and nectar.

Prior to about 1920 bee losses had been caused mainly by the application of insecticides to fruit trees. With cheaper calcium arsenate available, the dusting of cotton for the control of the boll weevil became practical (13), and a new source of poisoning for the honeybee resulted. In 1922 Neillie and Houser (37) reported the use of the airplane in applying insecticidal dusts. The airplane was adapted immediately for use in cotton dusting (14), and by 1926 it was being used extensively for pest control in many countries (31). In the same year Hilgendorff and Borchert (28) reported that the application of insecticides by airplane had raised protests from beekeepers, and that investigations had proved that serious losses by beekeepers were closely connected with such dusting. Eckert and Allinger (16) in 1935 reported the death of 3,000 colonies from crop dusting in one county in California, and as many more were rendered unproductive. Root (41) in 1940 reported that thousands of colonies had been killed in Texas and California by airplane dusting.

Whether increased losses resulted from use of the airplane or from the greater effectiveness or more extensive use of insecticides has not been determined. Eckert and Allinger (16) state that tomato fields had been dusted each season with ground dusting machines and not until the airplane was used were the bees noticeably injured. However, other reports show that losses have occurred in areas where only ground dusting machines were used.

The literature establishes the fact that insecticides drift from the fields over which they are applied, but the distance that the dust may drift is undetermined.

In applying insecticides to small plots Arant (3) and Smith, *et al.* (44) observed drift. Herbert (26) and Potts (40) recommend the use of sprays from an autogiro to reduce loss from drift. Thomas, *et al.* (47) have shown that dust applied by airplane may drift off the field and onto adjacent pasture land in quantities sufficient to cause sickness and death of cattle. Frederick (24) reported, however, that alfalfa dusted with calcium arsenate at the rates of 3 and 6 pounds per acre just prior to harvesting was fed to cattle, horses, and sheep for forty days without any ill effect. Evinius (21) and Marcus (35) recommended moving bees 3.5 miles from areas to be dusted. Himmer (29) placed the danger zone to bees for ground machine dusting at 3 miles and for airplane dusting at 6 miles.

McGovran, *et al.* (33) and Berthoff and Pilsen (5) have shown that particle size and toxicity are inversely related, the smaller particles being more toxic. It is well known that the smaller particles will stay in suspension in air longer and thus may be carried farther by the wind. Eckert and Allinger (17) exposed 18- by 30-inch plates 0.75 mile from a tomato field dusted with calcium arsenate and caught 221 micrograms of $Ca_3(AsO_4)_2$ per plate, equivalent to 0.49 microgram per square inch, enough to cause the death of honeybees.

Eckert (18) showed that bees separated by barren land from good sources of nectar flew 8.5 miles to the nectar. Even when the bees were in areas of relatively good flora in all directions, they foraged as far as 2.75 miles from the apiary, but the percentage of bees going that far was not determined. Root (41) observed bees collecting nectar from asters 5.0 miles from the apiary, but he concluded that as a general rule bees do not fly much more than 1.5 miles. That bees from different colonies in an apiary do not work the same source for food was also observed by Eckert (18). His observations indicate that a colony 8.5 miles or less from a dusted area might be poisoned if plants in other areas were unattractive to bees, whereas other colonies much closer might not be poisoned if the bees were foraging in another direction.

The normal amount of arsenic in bees, as well as the minimum lethal dose, has been studied by several workers. A small amount of arsenic appears to be a normal constituent of bees, as indicated by the scarcity of analyses failing to show presence of this element. That an exceedingly small amount of arsenic will cause the death of bees is also indicated. Furthermore, Hoskins and Harrison (30) have shown that sublethal amounts of arsenic will shorten the life of bees. McIndoo and Demuth (34), Cook and McIndoo (15), and Campbell (8) found a difference in death rate when different forms of arsenic were used. The arsenites are more toxic than the arsenates.

Fleming (23) found that temperature affected toxicity of lead arsenate to the Japanese beetle (*Popillia japonica* Newm.). Campbell (9) found that the age of the insect affects its susceptibility to poison. Schwartze (42) found arsenious oxide to be more toxic when finely ground that when the crystals were larger.

A scale of arsenic trioxide values classified in relation to their harmfulness to bees was prepared a few years ago by F. E. Todd, of the Bureau of Entomology and Plant Quarantine. This scale, slightly modified for the purposes of these investigations, is as follows:

Interpretation	Micrograms of As_2O_3
	Per bee:
Normal	0.15 or less
Contact with arsenic, probable life shortening	0.15 to 0.29
Definite poisoning	0.30 or more
	Per gram of pollen:
Definite poisoning	2.00 to 3.00 or more

There is little information in the literature on the toxicity of arsenic to bee brood. McIndoo and Demuth (34) analyzed dead larvae and pupae from poisoned colonies but did not find arsenic. Webster (51) and Herman and Brittain (27) found that brood died in connection with arsenic poisoning of the colony. Eckert and Allinger (17) found ten and thirteen parts per million of calcium arsenate in larvae and pupae, respectively.

Herman and Brittain (27) found from 2.2 to 98.0 micrograms of As_2O_3 per gram of pollen following orchard spraying, and they believed that pollen was the main source of poisoning in the hive. Mclndoo and Demuth (34) found as much as 151 micrograms of As_2O_3 per gram of pollen removed from bees' legs, and 120 micrograms per gram of fresh stored pollen; yet when they fed pollen that assayed only 1.57 and 3.39 micrograms of As_2O_3 per gram to caged bees the death rate increased over that of bees receiving nonpoisoned pollen. Various other workers have found large amounts of arsenic in the pollen following the application of arsenical insecticides near by.

Wodehouse (52), Pope (39), and Erdtman (19) have made extensive studies of pollen identification. Armbruster and Oenike (4), Zander (53), and Griebel (25) have studied pollen in honey in relation to determining honey source. With slight modification the methods developed by these workers have been followed in pollen identification studies reported in this paper,

EXPERIMENTAL METHODS

Practically all studies were made on dusts applied by airplane. Samples of bees, brood, and pollen were collected and observations made at every opportunity. To observe the most noticeable effect on bees it was essential that the experimenter be present at the time of the first application. This requirement often prevented his returning to former locations as often as desirable for continued observation and sampling.

Bees, larvae, or pupae were taken with forceps from the hive, 100 constituting a sample, and placed in tightly closed small glass jars until time for analysis. Pollen was either trapped at the hive entrance or removed from the cells of the comb. About 4 grams of fresh pollen constituted a sample.

The chemical analyses involved digestion of the material and determination of the arsenic content. The digestions were performed by use of a method recommended by Cassil (10).

The amount of arsenic was determined by the volumetric micromethod adapted by Cassil and Wickman (12) and extended by Cassil (11). The method is based upon the evolution of arsenic as arsine and the reduction of standard iodine solution in the conversion of arsine to arsenic trioxide.

Results of analyses of bees, larvae, and pupae are expressed in micrograms of As_2O_3 per individual, as the insects were counted rather than weighed. For consistency, values for pollen are stated as micrograms of As_2O_3 per gram which is numerically identical to the more common value, parts per million.

EXPERIMENTS COMPARING EFFECTS OF DIFFERENT ARSENICAL DUSTS

In 1944 and 1945 most of the dust applied to cotton in Arizona consisted of sulfur and Paris green in two different mixtures. The one most commonly used was generally referred to as the 7.5 per cent Paris green dust; the other dust contained 15 per cent of Paris green. The actual percentage composition of the Paris green dusts was as follows:

	7.5 per cent	15 per cent
Cupric acetoarsenite	6.38	12.75
Sulfur, 325-mesh	83.13	76.00
Inert	10.49	11.25

In one season observations were made on a proprietary mixture, also applied to cotton. This mixture, referred to as the 15 per cent calcium arsenate dust, consisted of the following ingredients (figures in per cent):

Tricalcium arsenate	9.45	
Calcium arsenite	0.82	
Inert	4.73	
Total	15.00	
Sulfur, 325-mesh		85.00

The application of other calcium arsenate mixtures and of calcium arsenate alone on young vegetables and of sulfur alone on citrus, onions, and beets was also observed.

PARIS GREEN 7,5 PER CENT

Figure 1 shows the gain in weight of a colony of bees and the arsenical analyses of samples of trapped pollen as affected by cotton dusting in an adjacent field. More than 1,000 acres of cotton within reach of these bees received two airplane applications of the 7.5 per cent Paris green dust at the rate of 20 pounds per acre.

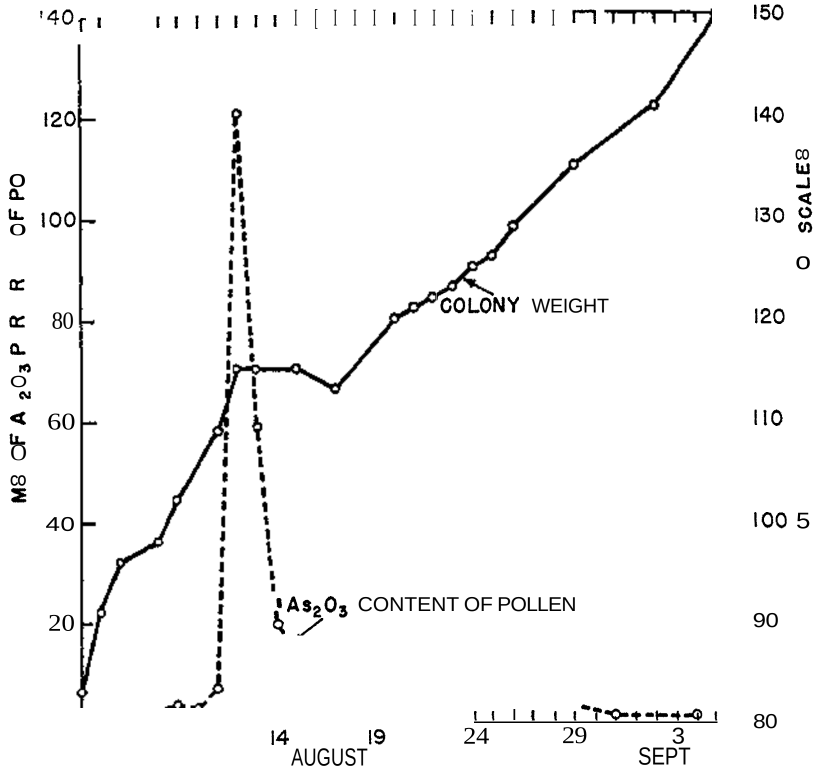

FIGURE 1.—As_2O_3 content of trapped pollen and weight of a colony of bees as affected by the dusting of cotton near by with the 7.5 per cent Paris green dust at the rate of 20 pounds per acre.

The cotton within approximately 0.5 mile of the hives was dusted on August 12 and 22, as indicated by the higher amounts of arsenic shown in the pollen analyses on these dates; however, dust was being applied almost daily within 3 miles of the bees from August 8 to 27. The source of the pollen showing the extremely high arsenical content of 120.4 micrograms of As_2O_3 per gram was identified as tamarix, a tree that grows along the canals and borders of the irrigated fields. Less than 10 grams of this pollen were obtained, whereas larger amounts of mixed pollens were trapped. These mixed pollens, none of which were analyzed separately, came from both desert plants and cultivated field weeds. If a large amount of the highly contaminated pollen had been stored, the colony would undoubtedly have been destroyed.

A better than average flow of honey was obtained, apparently because much of the cotton near the bees was of the long-staple variety, which yields more nectar, in Arizona, than the upland variety. No other source of nectar was available in appreciable quantity at this time. As indicated by the drop in weight (Fig.

1) of the scale colony, these colonies were affected by the dust application. Decreasing populations caused a decrease in honey production, but the colonies were not killed. This appeared to be the usual effect of the 7.5 per cent Paris green dust on the bees.

PARIS GREEN 15 PER CENT

Observations were made in an apiary close to a 100-acre cotton field that had received two airplane applications, a week apart, of the 15 per cent Paris green dust at the rate of 20 pounds per acre. Samples were taken from a normal colony when it was moved into this apiary four days before the second application was made, and again nine days after this application. The analyses, in micrograms of As_2O_3 per bee or per gram of pollen, were as follows:

	Before dusting	After dusting
Live bees from within the colony	0.11	0.46
Pupae alive, ready to emerge	0.06	0.05
Pupae dead, young	—	0.29
Pollen, stored in combs	1.83	—

No pollen was taken for analysis after the dusting, as there was no indication that additional pollen had been stored. The analyses show that the bees, pupae, and pollen contained only normal amounts of arsenic before the dust was applied, but nine days after the dust had been applied the bees and young pupae were poisoned. The old pupae that had been sealed in their cells when the dust was applied contained only normal amounts of arsenic.

This colony died a few days later from the effects of the dusting. The other colonies in the apiary likewise were either killed or seriously damaged. Various other apiaries near fields treated with this dust were observed, and the results were similar in most cases. Some apiaries, however, escaped serious injury, apparently because the bees were foraging in other directions.

CALCIUM ARSENATE 15 PER CENT

When the 15 per cent calcium arsenate dust was applied to cotton near apiaries, the colonies were either killed or seriously damaged. A sample of stored pollen from one such apiary contained 9.45 micrograms of As_2O_3 per gram, and dead larvae from the same hive contained 0.32 microgram of As_2O_3 per larva.

The vegetable fields are often treated with dusts containing much higher percentages of calcium arsenate. In an area where such applications were made during October, three samples of bees taken from an apiary about six weeks later showed 0.45, 0.50, and 0.55 microgram of As_2O_3 per bee, and samples of stored pollen showed 14.99, 11.01, and 38.10 micrograms per gram.

These analyses show that both stored pollen and the bees were highly contaminated with the arsenic. Many of the colonies in this apiary were dead, and the few remaining colonies were so severely damaged that they soon died.

Other samples were taken in the same general area (Table 1). These samples are grouped according to the location of the colony when the sample was taken. Some of the colonies had remained in the area since October, when the dust was applied; others had been moved 20 miles from the dusted area shortly after Christmas. For comparison, other samples were taken from apiaries that had never been exposed to dust. At the time the samples were taken from colonies in the dusted area, the colonies were weak and some were dying, and all the analyses showed that abnormal amounts of arsenic were still present. Movement of the colonies from the area after dust was applied did not reduce the arsenic content within the colony.

TABLE 1.—ARSENIC (As_2O_3) CONTENT OF SAMPLES OF BEES AND POLLEN (IN MICROGRAMS PER BEE) TAKEN FROM APIARIES SIX MONTHS AFTER EXPOSURE TO CALCIUM ARSENATE DUST IN COMPARISON WITH THAT OF BEES AND POLLEN NOT EXPOSED

Location of colony	Live bees	Live pupae	Stored pollen
In dusted areas entire time	0.13 0.18	0.11	6.12 2.46
Moved from area three months after dusting	0.11 0.13 0.14		4.34
Never exposed to dust	0.07 0.07	0.08	1.43 1.57

REPELLENCE OF SULFUR TO HONEYBEES

The repellent effect of sulfur was determined by making many observations on relative abundance of honeybees in dusted and nondusted fields. During the cotton dusting season in Arizona temperatures above 100 degrees F. are common. Occasionally during the hottest periods of the day bees seemed to be slightly less numerous in dusted cotton fields and in alfalfa fields over which the dust had drifted than in similar nondusted fields. Observations in and near a citrus grove dusted by airplane with sulfur at the rate of 100 pounds per acre revealed a heavy population of bees in both the dusted and nondusted areas. There was no repelling effect, nor was there any indication of damage to near-by colonies as a result of the dusting. At the time of the application the temperature was 78 degrees F. and the relative humidity 18 per cent. The conclusion was reached that the repelling effect of sulfur on bees was of no practical importance.

EFFECTS OF DIRECT EXPOSURE TO DUSTING

SCREENED CAGES OF BEES EXPOSED TO DUST

For observations on the effect of the dust on caged bees four screen-wire cages, of a type commonly used by commercial beekeepers in shipping 2 pounds of bees by express, were supplied with a sugar-water solution and 1 pound of bees each. Two of these cages, designated as A and B, were placed in a cotton field before being dusted with the 7.5 per cent Paris green dust at the rate of 15 pounds per acre. The other two cages, designated as C and D, were placed in a nondusted cotton field 5 miles away. As soon as the dusting was completed, about 10 A.M., the positions of cage A and C were interchanged; that is, cage A was moved to the dust-free field and cage C to the dusted field. In this manner any reaction to either contact of the dust particles or fuming of the sulfur could be observed. The cages were placed slightly above the tops of the cotton plants and, to afford protection from direct rays of the sun, 16- by 24-inch shade boards were placed over each cage after the dust had been applied.

Daily maximum temperatures during this experiment exceeded 100 degrees F. The cages were observed every few hours on the first day, and then daily for eight days, when the experiment was discontinued. At the end of the eight days, out of an estimated 4,000 bees per cage, cage A had 212 dead bees, cage B 192, cage C 125, and cage D 225. The bees kept well-formed clusters at all times, and no differences could be observed in their activity. Apparently neither the amount of dust that came in contact with the bees nor the sulfur fumes were toxic to them.

SCREENED AND OPEN COLONIES EXPOSED TO DUST

The effectiveness of caging colonies for the day on which the dust was applied was determined by placing two colonies near a 60-acre cotton field to be dusted with 15 pounds per acre of 7.5 per cent Paris green dust. A screen-wire cage was placed over one of the colonies at dawn of the day dust was applied. Water was supplied this colony through a Boardman entrance feeder. Bees of the other colony were not restricted from flying. The cage was removed at the end of the day. Some dead bees were on the ground inside the enclosure. Both colonies were observed for a week, but neither showed abnormal loss in population. Particles of Paris green were found in a petri dish on one of the hives, although the dust cloud drifted in the opposite general direction from the field.

This test was repeated in another area, where 7.5 per cent Paris green dust was applied twice, a week apart, to a 40-acre cotton field. The first application was at the rate of 15 pounds per acre; the second at the rate of 20 pounds per acre. About a pound of bees died each day that a colony was caged. An analysis showing only 0.13 microgram of As_2O_3 per bee proved that the bees were not killed by the dust. A similar quantity died when a colony in a dust-free area was caged. Since no great difference between the caged and open colonies was discernible at any time, no value was obtained from this test.

COLONIES DUSTED BY AIRPLANE

Two colonies were used to study the effect of dusting colonies by airplane. By request the pilot dusted the colonies three times from an altitude of about 10 feet, applying 7.5 per cent Paris green dust at 20 pounds per acre. The plane passed directly over the colonies once, about 10 feet to windward once, and about 10 feet leeward once, although the hives were enveloped in the swirling dust cloud each trip. Examination of the hive cover with a hand lens immediately after the dust was applied revealed an even cover of dust, and bees taken from the lighting board had particles of Paris green on the hairs of their bodies. The colonies were observed for several months, but no effect of exposure to the dust was observed.

EFFECT OF DRIFTING DUST

Two colonies were placed at one end of a 320-acre cotton field, 1.0 x 0.5 mile, to be dusted with 15 per cent calcium arsenate at the rate of 20 pounds per acre. Each hive was ventilated at the top by a 4-inch frame with a screen-wire between the hive cover and the upper super of combs. The entrance to one hive was left open, but the entrance to the other was closed with screen wire. The dust drifted lengthwise over the field, and the colonies were in direct line of the maximum drift. The dusting operation required two hours, and dust continued to drift from the field for several minutes.

After the dust cloud had completely disappeared, the closed hive was returned to its home location 15 miles away, and the entrance opened. The colony was observed for several months, but at no time were any effects noted of exposure to the drifting dust. The condition of the colony indicated that dust neither drifted nor was pulled into the hive by the ventilating bees in amounts sufficient to cause damage.

A second application of the same type of dust was similarly applied seven days later. The colony in which the bees were free to fly was left in the dusted area for ten days. At the end of that time the colony strength was reduced at least by half. The colony was then returned to the home location, where it weakened rapidly.

The bees were all dead thirty-eight days after first exposure to the dust. The test proved that drifting of this dust over an apiary does not cause the damage, but that death is caused by the bees getting the poison in the field and bringing it to the hive. Analyses of samples taken from the open colony (Table 2) show that the bees, larvae, and pollen were contaminated with arsenic, although the content was comparatively low in the pollen.

Honey was not analyzed, as other workers (34, 46, 51) have found only a trace or no arsenic in honey, and it has not been considered generally to be a source of poisoning. Most beekeepers believe that if bees collect poisoned nectar they die either before returning to the hive or before converting the nectar into honey.

TABLE 2.—ANALYSES OF SAMPLES TAKEN FROM A COLONY EXPOSED TO DRIFTING 15 PER CENT CALCIUM ARSENATE DUST AND THEN LEFT IN THE DUSTED AREA FOR TEN DAYS

Type of sample	Days after dusting		Micrograms of As_2O_3 per bee
	First application	Second application	
Dead bees, at entrance	5		0.85
Live bees, coated with cotton pollen taken at entrance	5		0.35
Live bees, picked off combs	9	2	0.40
Live larvae, full grown	9	2	0.14
Stored pollen, Johnson grass and alfalfa	9	2	1.6[a]
Stored pollen, mostly Johnson grass	37	30	1.0[a]
Live bees, picked off combs	37	30	0.29

[a] Per gram

EFFECT OF WIND VELOCITY AND TEMPERATURE AT TIME OF DUSTING

The literature reveals only a few vague references to the effect of velocity of the wind on dusting, other than general expressions such as "light breeze," "strong breeze," and "windy." Escherich (20) concluded that it was useless to dust in wind of more than 2 or 3 meters per second (4.5 to 6.7 miles per hour) or in sunshine that caused strong vertical currents. Thomas, *et. al.* (47) noted that airplane dust applications were made "until the wind would interfere and prevent satisfactory application of the dust," although they report one instance of dust being applied to a 500-acre field when the breeze was so strong that the dust was blown half the width of the field and much of it drifted into an adjacent pasture. The airplane crop dusters use such criteria as those listed in the Beaufort scale, Albright (2), of wind velocities (Table 3), as well as observations on the movement of the cloud of applied dust, to determine the effect of wind on the results of their work.

Figure 2 shows wind velocities recorded during August, 1945, at various cotton fields where observations were being made on amount and extent of drift while the airplanes were applying dust. The anemometer was set up in open areas, the cups being about 6 feet from the ground. A register attachment was not used, but the dial was read frequently during dusting operations. The wind velocities for the dates and hours indicated ranged from 1.0 to 7.2 miles per hour, but most of the work was done when they were between 2 and 4 miles per hour. As indicated by Twenhofel (49), increased velocities would have been obtained if the anemometer had been located higher above ground. Blacktin (7) states that a wind of 1.75 miles per hour will move particles from 2 to 200 microns in diameter; therefore the wind velocities recorded were sufficient at practically all times to carry the dust particles being applied. This was verified by the observation that there was always some drift from the dusted field.

TABLE 3.—BEAUFORT SCALE OF WIND VELOCITIES[1]

Number	Descriptive term	Velocity, miles per hour	Land criteria
0	Calm	Less than 1	Calm, smoke rises vertically.
1	Light air	1 to 3	Direction of wind shown by smoke drift, but not by wind vanes.
2	Light breeze	4 to 7	Wind felt on face; leaves rustle; ordinary vane moved by wind.
3	Gentle breeze	8 to 12	Leaves and small twigs in constant motion; wind extends light flag.
4	Moderate breeze	13 to 18	Raises dust and loose paper; small branches are moved.
5	Fresh breeze	19 to 24	Small trees in leaf begin to sway; crested wavelets on inland water.
6	Strong breeze	25 to 31	Large branches in motion; whistling heard in telegraph wires.
7	Moderate gale	32 to 38	Whole trees in motion; inconvenience felt when walking against wind.
8	Fresh gale	39 to 46	Breaks twigs off trees; generally impedes progress.
9	Strong gale	47 to 54	Slight structure damage occurs (chimney pots and slate removed).
10	Whole gale	55 to 63	Seldom experienced inland; trees uprooted; considerable damage.
11	Storm	64 to 75	Very rarely experienced; accompanied by widespread damage.
12	Hurricane	Above 75	

[1]After Albright (2).

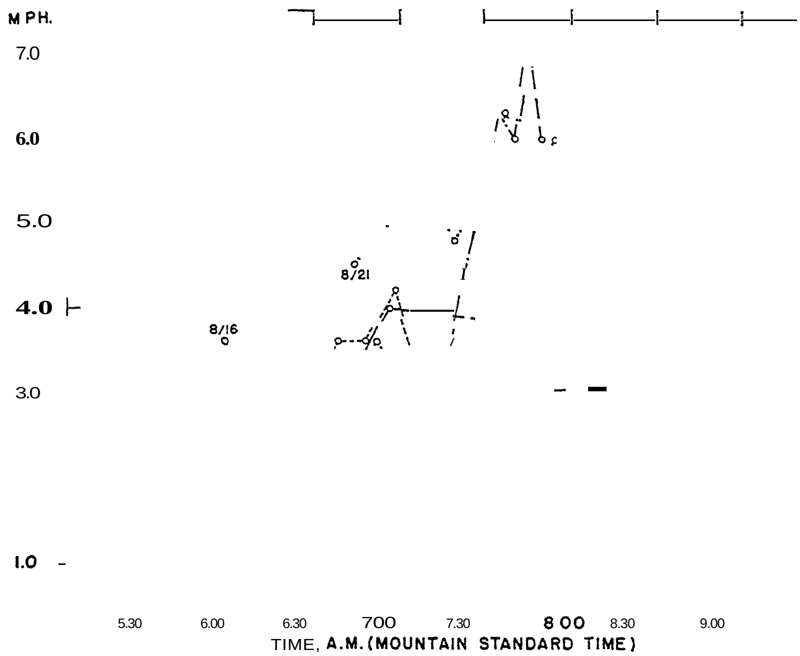

Figure 2.—Wind velocities at time of arsenical dust applications in cotton fields during August, 1945. Each line indicates the velocity for the time and date shown.

The recorded temperatures during the above dusting operations are presented in Figure 3. These temperatures ranged from 65 degrees to 94 degrees F., and were recorded between 5:30 and 9:10 A.M., Mountain standard time. With increasing temperature the dust had a decreasing tendency to settle. When temperatures of 90 degrees F. or above were recorded, a considerable amount of the dust was observed to rise and disappear in the air. Whether this type of drift was more or less dangerous to bees than the low-hanging dust cloud was not determined.

METHODS OF DELIMITING AREAS OF DRIFT

DUST CLOUD OBSERVATIONS

Eckert and Allinger (17), in California, reported that clouds from calcium arsenate-dusted fields could be seen clearly 2 to 3 miles from the point of application. Three miles was the maximum distance from Arizona fields at which a dust cloud was definitely discernible at right angles to the direction of drift. In such qualitative observations no distinction was made between the arsenical in the dust and the accompanying sulfur or inert material, nor was the percentage of the applied dust observed at the different distances.

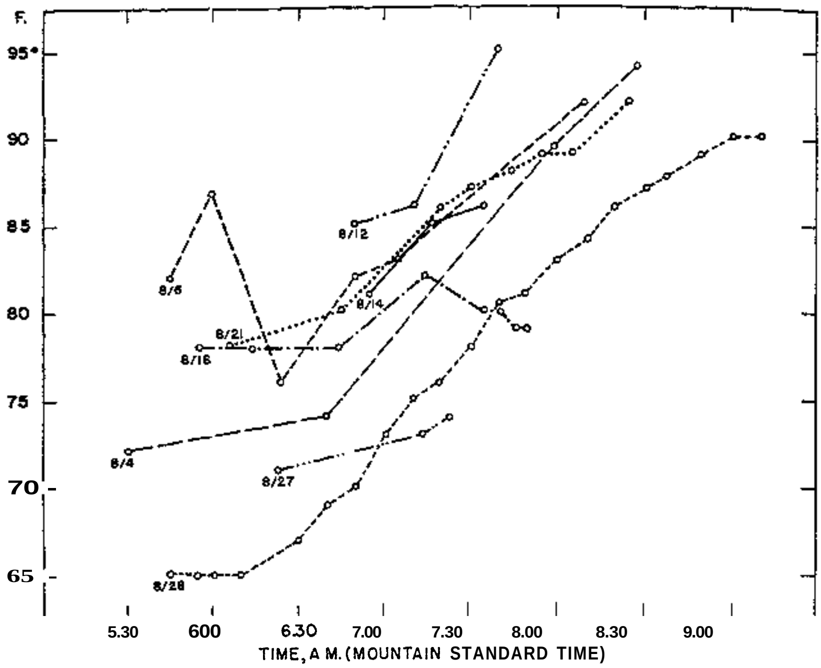

Figure 3.—Temperatures at time of arsenical dust applications in cotton fields during August, 1945. Each line indicates the temperature for the time and date shown.

IDENTIFICATION OF DUST PARTICLES

The areas being covered by the drift were also partially determined by the identification of the Paris green particles with a hand lens which magnified ten times. This method was rapid but could only be used on particles having distinctive colors or shapes, such as the Paris green particles. These particles could easily be found on leaves and blossoms in fields adjoining the dusted fields. On several occasions Paris green particles were found more than a mile from the dusted fields, and chemical analyses of material deposited in exposed petri dishes showed arsenical deposits as far as 2.25 miles.

CHEMICAL ANALYSES

Two methods were employed in the collection of samples for analysis to delimit the areas of drift. Eight-inch aluminum petri dishes were exposed at various distances from dusted fields for collection of dust deposits. Samples of bees, brood, and pollen also were taken.

Dust deposits in exposed petri dishes

A hypothetical composite area showing a field dusted with Paris green and the direction and amount of drift, as indicated by analy-

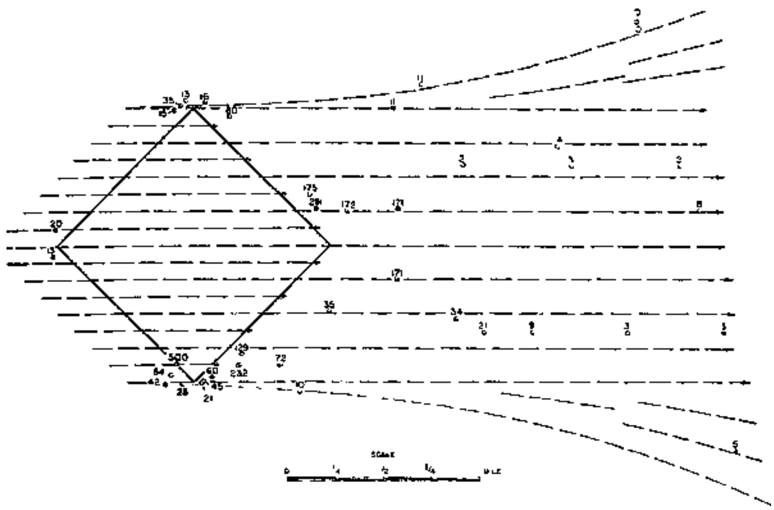

Figure 4.—Hypothetical composite area, showing location of petri dishes exposed to Paris green dust drifting from a dusted field. The numerals represent the micrograms of As_2O_3 recovered from individual dishes, recalculated to a standard dosage of 20 pounds per acre of the 7.5 per cent dust. Arrows indicate direction of drift from the square area which represents the dusted field.

sis of dust deposits in exposed petri dishes, is diagramed in Figure 4. As most of the samples were taken near fields dusted with the 7.5 per cent Paris green dust at 20 pounds per acre, all the other samples are recalculated on this basis. Theoretically, at this rate of application, 3,032 micrograms of As_2O_3 should be deposited on an area the size of the petri dish, provided there is no drift from the dusted field.

It is recognized that analyses of deposits in a large number of petri dishes placed within the fields would have given a more practical average value than the theoretical value of 3,032 micrograms. At the time the samples were being collected, however, the interest was entirely upon amount and distance of drift beyond the limits of the fields. To evaluate the drift from the many fields examined, the hypothetical field was devised and the theoretical amount of arsenic per dish was calculated. These data, summarized in Table 4, indicate a rapid decrease of As_2O_3 with distance from the field, but show some drift as far as 2.25 miles.

Analyses of bees, pollen, and brood

Bees *collected on plants in adjoining field.*—A sample of live nectar-collecting bees taken in an alfalfa field the day an adjoining cotton field was dusted with the 7,5 per cent Paris green at the rate of 15 pounds per acre averaged 1.12 micrograms of As_2O_3 per bee. A similar sample collected on the same day in alfalfa 1.5 miles away, but in the direction the dust drifted, contained only

TABLE 4.—ACTUAL DEPOSITS OF As_2O_3 IN PETRI DISHES AT VARIOUS DISTANCES FROM DUSTED FIELDS COMPARED WITH THE THEORETICAL DEPOSITS IN THE FIELDS OF 3,032 MICROGRAMS OF As_2O_3 PER DISH

Miles from field	Micrograms of As_2O_3 per dish	Percentage of theoretical deposit
0 (border of field)	500	16.5
0 to 0.25	13 to 291	0.4 to 9.6
0.25 to 0.50	10 to 171	0.3 to 5.6
0.50 to 0.75	11 to 34	0.4 to 1.1
0.75 to 1.00	2 to 25	0.07 to 0.8
1.00 to 1.25	3 to 14	0.1 to 0.5
1.25 to 1.50	3 to 8	0.1 to 0.3
1.50 to 1.75	2 to 6	0.07 to 0.2
1.75 to 2.00	10	0.3
2.00 to 2.25	5	0.2

0.16 microgram. Three days later another sample collected in the alfalfa adjoining the dusted field contained 0.62 microgram.

Bees collecting nectar from the cotton on the day the dust was applied at 20 pounds per acre contained 1.45 micrograms of As_2O_3 per bee; bees taken in the same field sixteen days later contained 0.59 microgram, and sixty days later 0.08 microgram. A sample from a nondusted cotton field contained 0.09 microgram of AS_2O_3 per bee.

Bees collecting nectar from tamarix alongside the cotton, but not directly in the line of drift, on the day the same dust was applied contained 0.49 microgram of As_2O_3 per bee. Another sample taken at the same place forty-five days later contained 0.16 microgram.

These analyses indicate that, although the dust drifted onto adjacent alfalfa and tamarix in quantities sufficient for bees to collect lethal amounts, it did not drift to the alfalfa field 1.5 miles away in quantities sufficient to cause damage to bees.

Examination of live bees with a hand lens often revealed particles of Paris green on them or on the loads of pollen they were carrying if the bees had been foraging in dust-treated territory. In one instance particles of Paris green were found on working bees in a cotton field five days after the dust had been applied.

Bees taken from hives or near hive entrances.—Samples of bees were commonly taken in which As_2O_3 content far exceeded the minimum lethal dose of 0.30 microgram. Several samples of dead bees near hive entrances contained more than 1.00 microgram. One sample of such bees contained on an average 1.03 micrograms of As_2O_3 per bee. Vegetables about 1 mile away had been dusted with calcium arsenate, and all the colonies in the apiary were killed. Another sample, consisting of bees unable to fly but crawling on the ground near hive entrances, contained on an average 1.38 micrograms of As_2O_3 per bee. Cotton less than 0.5 mile away had been dusted with the 15 per cent Paris green dust, and the apiary was destroyed.

Several samples of bees taken from within the hive failed to show any symptoms of poisoning, and yet the analyses revealed more than 0.50 microgram of As_2O_3 per bee. In all such instances the colonies ultimately were seriously affected. A study of the source of 124 samples of bees in relation to their arsenical content revealed that the toxicity scale is a dependable measure in determining poisoning in bees, whether they were taken from within the hive or picked up crawling or dead at the entrance. However, bees might contain much more than 0.30 microgram of As_2O_3 per bee for some time before they died. That all bees do not die at once from heavy doses of arsenic is indicated by a laboratory test in which caged bees were fed sugar sirup having a high Paris green concentration (1,000 micrograms per gram). The mortality of poisoned bees after twenty-four hours was 47 per cent, after forty-eight hours 72 per cent, after seventy-two hours 93 per cent, and after ninety-six hours 100 per cent. No analyses were made of these bees.

Bees taken from scattered points away from dusted area.—Thirty-seven samples of live bees were taken from colonies at widely scattered points throughout Arizona and more than 3 miles from any known arsenical dusting. With the exception of four samples taken near smelters and containing from 0.15 to 0.39 microgram of As_2O_3 per bee, all the samples contained between 0.02 and 0.13 (average 0.08) microgram.

Of seven samples of live bees taken between 2 and 3 miles from known dusting, five contained arsenic ranging from 0.06 to 0.13 (average 0.09) microgram of As_2O_3 per bee. One sample contained 0.16 microgram, and one sample collected near the hive entrance 2.5 miles from a cotton field dusted with the 15 per cent calcium arsenate dust averaged 0.75 microgram. More than half of the colonies in the apiary from which this sample was taken died as a result of this single application of dust.

Of nineteen samples of live bees taken from colonies between 1 and 2 miles from dusted fields, eight samples contained normal amounts of arsenic, ranging from 0.04 to 0.15 (average 0.07) microgram of As_2O_3 per bee; five samples probably were poisoned, having an arsenic content ranging from 0.16 to 0.27 (average 0.20) microgram; and six samples were definitely poisoned, ranging in arsenic content from 0.32 to 1.42 (average 0.66) micrograms.

Of sixty-one samples of live bees taken from colonies within 1 mile of dusted fields, ten samples have normal amounts of arsenic, ranging from 0.08 to 0.14 (average 0.11) microgram; twenty probably were poisoned, having an arsenic content of 0.15 to 0.29 (average 0.22) microgram; and thirty-one samples, ranging in arsenic content from 0.31 to 1.38 (average 0.62) micrograms, were definitely poisoned.

Pollen taken from scattered points away from dusted area.—Twenty-three samples of stored pollen were also taken from widely scattered points throughout Arizona and more than 3 miles from any known arsenical dusting. With the exception of one sample taken near a smelter and containing 5.1 micrograms of

As_2O_3 per gram, all the samples contained between 0.5 and 2.0 (average 1.4) micrograms of As_2O_3 per gram of pollen. Two samples collected between 2 and 3 miles from known arsenical dusting contained identical amounts of arsenic, namely, 1 microgram.

Of twenty-four samples of pollen taken from combs of colonies between 1 and 2 miles from known arsenical dusting, two samples were definitely poisoned, containing 6.6 and 6.8 micrograms of As_2O_3 per gram of pollen; four samples probably were poisoned, having an arsenic content ranging from 2.3 to 2.8 micrograms; and the eighteen other samples, ranging in arsenic content from 0.7 to 1.9 (average 1.1) micrograms, were not considered poisoned.

* Of forty-nine samples of pollen taken within 1 mile of fields known to have been dusted with arsenicals, only sixteen were considered to have normal amounts of arsenic, ranging from 0.5 to 1.8 (average 1.3) micrograms per gram; eleven samples were considered probably poisoned, the arsenic content ranging from 2.0 to 4.0 (average 1.3) micrograms per gram; and twenty-two samples were considered definitely poisoned, ranging in arsenic content from 4.2 to 120.4 (average 21.4) micrograms per gram. Practically all the pollen samples were dug from combs in colonies; however, a few were trapped from bees' legs at the colony entrances.

These analyses show that the greatest poisoning of bees and pollen occurred within 1 mile of dusted fields, with some poisoning between 1 and 2 miles, one instance at 2.5 miles, and no evidence of poisoning beyond that point.

One sample of pollen trapped from a colony in a nondusted area contained pollen pellets of three such distinct colors that they were easily separated. One group of pellets was identified as coming from Johnson grass, one from rayless goldenrod, and the third was not identified but was distinctly different from the other two. Each group assayed identical amounts of arsenic, 0.8 microgram of As_2O_3 per gram, an indication that the plant source of the pollen does not account for the great difference in arsenic content between different samples.

Some of the pollen samples were taken the day the dust was applied; others, including samples from the hives, at various times up to six months after dust was applied. The analyses indicate that time is not an important factor, in that high arsenic content may be found in stored pollen for months after dust is applied, provided the same pollen is still present. Analyses of samples of bees and pollen from the same apiary show a high correlation between arsenic in the bees and in the pollen.

Brood *taken from hives.*—Analyses of thirty-four samples of larvae and pupae indicate that, although some variations can be expected in samples, an As_2O_3 content of more than about 0.1 microgram per individual indicates poisoning. Larvae that had not completed their feeding period might show less arsenic than older larvae. Pupae that were sealed when the colony was exposed to the poison might show no abnormal amount. Many of the pupae that showed abnormal amounts of arsenic were dead when removed from the cells. Dead and deserted pupae scattered in the cells of the comb may indicate arsenic poisoning.

The As_2O_3 content of six samples of normal larvae ranged from 0.03 to 0.05 (average 0.04) microgram and of seventeen samples of normal pupae from 0.03 to 0.08 (average 0.05) microgram. The As_2O_3 content of six samples of poisoned larvae ranged from 0.07 to 0.32 (average 0.16) microgram and of five samples of poisoned pupae from 0.09 to 0.29 (average 0.18) microgram.

POLLEN IDENTIFICATION

Beekeepers acquainted with the nectar-producing plants in their vicinities can examine honey for color, viscosity, taste, and a few other qualities, and by knowing what plants are in bloom at the time within flight range of the bees can often deduce the plant source. An accurate method for determining honey source from nectar alone does not exist; however, pollen grains, when present in the nectar, have characters that permit determination of source with a fair degree of accuracy.

Plate I shows comparative shape and size of some of the pollen grains stored by bees in the irrigated sections of Arizona during the cotton-dusting season.

Bee-collected pollen was identified by comparison of pollen grains in the sample with grains taken either from anthers of identified plants or from pollen baskets of bees working on identified plants. The grains were mounted for preservation on glass slides.

The plant source was determined in most of the pollen samples analyzed. Pollen from mesquite, *Prosopis juliflora* (Swartz) DC., occurred in many of the samples taken just before the cotton was dusted, and pollen from Johnson grass, *Sorghum halepense* (L.) Pers., and alfalfa, *Medicago sativa* L., in many of those taken during the dusting season. Puncturevine, *Tribulus terrestris* L., was also a common source. Pollen from tamarix, *Tamarix aphylla* L., appeared in samples collected later in the dusting season, and rayless goldenrod, *Aplopappus* sp., in samples collected shortly after the cotton-dusting season ended and during fall vegetable dusting. Johnson grass and telegraph plant, *Heterotheca subaxillaris* (Lam) Britt and Rusby, are also common at that time.

As shown by Vansell (50), bees gather little cotton pollen in pellet form, but often return to the hive with the body hairs covered with it. Such pollen is not stored in the cells as are pollen pellets. This indicates that the high arsenic content of many of the samples of pollen analyzed was not due to contaminated cotton pollen. Johnson grass, however, yields large quantities of pollen, is common throughout the areas where crops were dusted, blooms throughout the warm season, and its anthers open sufficiently early in the morning to become contaminated by the early morning dust applications. Analyses of many samples consisting predominantly of Johnson grass pollen showed high arsenic content.

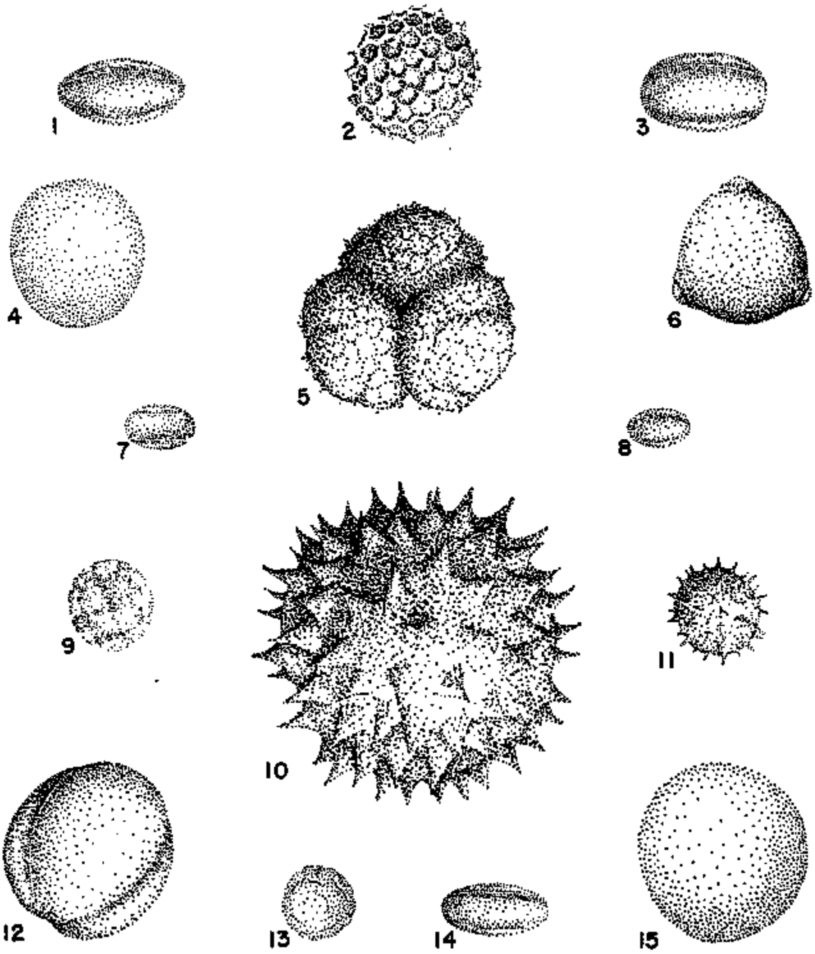

PLATE I.—Enlarged drawings of pollen grains common in Arizona during the cotton-dusting season.

No.	Scientific Name	Common Name	Size in Microns
1	*Prosopis juliflora* (Swartz) DC.	Mesquite	47
2	*Tribulus terrestris* L.	Puncturevine	47
3	*Medicago sativa* L.	Alfalfa	43
4	*Sorghum halepense* (L.) Pers.	Johnson grass	43
5	*Chilopsis linearis* (Cav.) Sweet	Desertwillow	73
6	*Cucumis melo* L.	Cantaloupe	50
7	*Larrea tridentata* (DC.) Coville	Creosotebush	24
8	*Tamarix gallica* L.	Tamarix	21
9	*Acacia greggii* A. Gray	Catclaw	33
10	*Gossypium hirsutum* L.	Upland cotton	114
11	*Helianthus annus* L.	Sunflower	36
12	*Cereus giganteus* Engelm.	Saguaro	72
13	*Atriplex canescens* (Pursh) Nutt	Saltbush	24
14	*Physalis wrightii* A. Gray	Ground cherry	37
15	*Sorghum vulgare* Pers.	Milo maize	83

DISCUSSION

The observations and data show that the arsenic in the insecticidal dust is the material that causes the damage to honeybees, and that the greater the percentage of As_2O_3 the more serious is the damage to the colonies. Where sulfur was used alone there was no discernible damage to bees, and the least damage occurred where the greatest percentage of sulfur was included in the insecticide.

During a preliminary survey of the area in which the observations were made, drift of the insecticides was a subject much discussed by beekeepers, farmers, and crop dusters. Beekeepers often mentioned drift of the dust over their apiaries as a cause of the damage, and objected to the crop-dusting plane flying over the apiary lest some of the dust be applied directly to the hives. Crop dusters offered the theory that only the light, inert material floated away from the dusted field.

The evidence shows that there was drift of the arsenical as well as of the inert portion of the dusts from the dusted fields, that the drifting dust about the hives did not cause damage, but that when the material settled on blossoms around the dusted fields bees could obtain poison from plants contaminated by the drifting dust. This condition was well established in connection with the dusting of young lettuce in the fall. The fields were free of weeds when the dust was applied, and the plants were small, having only a few leaves. When these fields were dusted, however, they were often surrounded by various fall-blooming plants, and pollen from these plants always appeared in the samples from near-by poisoned hives. Differences of opinion among beekeepers as to the effect of dust applications on their bees may therefore have been due to the type of dust applied in their area.

The danger zone around dusted fields beyond which bees may be safe was not determined; in all probability it varies with each location. The amount of material deposited on exposed petri dishes, the number of particles of Paris green found on plant material, and the arsenic found in bees foraging in fields adjoining dusted fields indicated that where the 7.5 per cent Paris green dust was applied bees might obtain a lethal dose of arsenic as far away as 0.5 mile. This zone may be extended considerably further, when calcium arsenate is used for the following reasons:

1. Calcium arsenate is lighter than Paris green.
2. The particles of calcium arsenate are much smaller than those of Paris green.
3. Dusts applied to vegetables contain a higher percentage of As_2O_3 than dusts applied to cotton, where most of the observations were made.

One apiary was severely damaged, although it was 2.5 miles from the dusted field and the dust drifted in the opposite direction, an indication that the bees flew 2.5 miles in sufficient numbers to obtain enough poison to cause the damage observed. The drift of the dust in addition to the flight of the bees would extend this danger zone to more than 3 miles. That apiaries were occasionally undamaged although closer to dusted fields indicates that the bees were not visiting the dusted plants or the plants over which the dust drifted.

Although arsenicals have been applied to vegetables in Arizona for several years, little damage to bee colonies was reported prior to 1944. Whether the increased damage was due to the increased amount of arsenicals used, the increased acreages involved, or the method of application was not determined.

No detrimental effect from contact of the arsenic particles with the external parts of the bee body was detected. Bees in their cleaning habits would tend to remove such particles from the hairs of their bodies, although particles of Paris green were sometimes found on bees in fields dusted with the Paris green-sulfur dust.

O'Kane and Glover (38) obtained evidence that arsenic confined for ten days in contact with the integument on the back of the cockroach affected that insect. King and Rutledge (32) killed adult locusts by allowing them to flutter for 20 seconds in a dust cloud of sodium arsenite. The tracheae contained arsenic, but no evidence was found of penetration through the integument. The spiracles of the bee are shown by Snodgrass (45) to be as large as 60 by 230 microns, whereas 95 per cent of all the Paris green particles applied were reported to be less than 45 microns in diameter (325 mesh), and the calcium arsenate particles were still smaller; therefore, the spiracles are sufficiently large to permit entrance of the dust particles. The lack of injury to the caged colony sitting in the drifting cloud of calcium arsenate dust indicates that inhalation of the particles is not the method by which the bees are poisoned.

Both the field observations and the analyses indicate that damage to bee colonies was inflicted only when the bees were foraging either on plants dusted with the arsenical or on plants to which the material drifted.

Although there were occasional exceptions, generally the arsenic content of stored pollen and of bees from the same colony followed similar trends. This was true whether the samples were taken a few days or several months after arsenical dust was applied.

SUMMARY

As a consequence of reported heavy losses of bees resulting from the application of insecticides to field crops in Arizona, a study was initiated to determine the cause and extent of such losses. This paper discusses bee poisoning with arsenicals, drift of applied arsenicals, normal and lethal doses of arsenic per bee, arsenical content of pollen and bee brood, and the value of pollen identification in connection with such a study.

Screened cages of bees exposed in cotton fields to which a dust containing 7.5 per cent of Paris green with sulfur as the diluent was applied showed that bees were not killed by exposure to the

applied dust or to the sulfur fumes.

Colonies dusted directly by airplane with the 7.5 per cent Paris green dust were not damaged.

A colony was not injured when exposed to drifting dust from a 320-acre cotton field dusted at 20 pounds per acre with 15 per cent calcium arsenate dust. This colony was given sufficient ventilation, but the hive entrance was screened to prevent flight until after the dust had drifted away, and then the colony was moved to another area before the bees were released. Another colony in a hive left open during the time of the drifting dust and in the same location for the next ten days was killed.

Evidence showed that poisoning by the 7.5 per cent Paris green dust was less severe than by either a 15 per cent Paris green or a 15 per cent calcium arsenate dust.

Observations indicated that the sulfur in the dust did not damage bees and had little or no repellent effect on them.

Dust clouds were observed as far as 3 miles from the fields over which the dust was released. Particles of Paris green were found more than a mile from the dusted field, and analyses of deposits on exposed petri dishes showed drift as far as 2.25 miles.

Bees collected in cotton fields sixteen days after a 7.5 per cent Paris green dust had been applied had high arsenical content, but similar samples taken sixty days after dusting showed only normal amounts of arsenic. Live bees collected on both tamarix and alfalfa near cotton fields dusted with the 7.5 per cent Paris green also were high in arsenic, but the arsenic content of live bees collected on alfalfa 1.5 miles from this dusted cotton, although in line of the drifting dust, was only slightly above normal.

Samples of bees that were collected 3 miles or more from known dusting contained not more than 0.13 micrograms of As_2O_3 per bee, with the exception of samples taken near smelters.

One sample of live bees, crawling near the hive entrance, averaging 0.75 microgram of As_2O_3 per bee, was collected 2 to 5 miles from a field dusted with the 15 per cent calcium arsenate dust. More than half the colonies in this apiary died as a result of this single dust application.

Samples of pollen collected 3 miles or more from known dusting contained not more than 2.0 micrograms of As_2O_3 per gram, with the exception of one sample taken near a smelter.

Two samples of definitely poisoned pollen were taken from combs of colonies more than 1 mile from fields dusted with the arsenicals.

More than 0.1 microgram of As_2O_3 in larvae or pupae and 0.3 microgram in adult bees indicated poisoning.

The arsenic content of bees continued to be high for months in hives containing stored pollen of high arsenic content.

Analyses of samples of bees and pollen from the same apiary showed a high correlation between arsenic content of the bees and that of the pollen.

Wind velocities at 6 feet above ground ranged from 1.0 to 7,2 miles per hour and generally were between 2 and 4 miles per hour at the time of dust applications. There was always some drift from dusted fields. Temperatures ranged from 65 degrees to 94 degrees F. With increasing temperature the dusts had a decreasing tendency to settle. At 90 degrees F., or above, a considerable amount of the dust was observed to rise and disappear in the air.

Identification of pollen collected by bees was of aid in determining where the bees had been foraging. Johnson grass, puncturevine, and alfalfa were the most common pollens found in the samples analyzed. Little cotton pollen was seen in the hives, and none appeared to have been stored in pellet form.

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