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EFFECTS OF NICOTINE SULPHATE AS AN OVICIDE AND
LARVICIDE ON THE CODLING MOTH AND THREE
OTHER INSECTS.

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INTRODUCTION.

For several years nicotine and its compounds have been used against certain soft-bodied insects as contact insecticides, and within the past few years the question has been raised concerning the effects of nicotine sulphate upon the eggs and early instars of other insects which are commonly controlled by other means.

During 1915 and 1916, in the State of Washington, De Sellem (1)² carried on field experiments with nicotine sulphate and arsenate of lead and claimed that the former insecticide was as efficient as the latter one for controlling the codling moth (*Laspeyresia pomonella* L.). During the season of 1917 similar experiments in three orchards were performed by the same author and others (2) on a larger scale in the same State, and the results obtained showed that nicotine

¹ Resigned March 31, 1920.

² Numbers (italic) in parentheses refer to "Literature cited," p. 18.

sulphate did not control the codling moth as well as did lead arsenate, and that its control was unsatisfactory on all of the plats sprayed, except one. The Bureau of Entomology also conducted some field experiments during the season of 1917 in three important apple regions in the United States, the first of these being in the Lake region at Benton Harbor, Mich.; the second one in the mountainous region at Grand Junction, Colo.; and the third one in the semiarid region at Roswell, N. Mex. In the first region the experiments were carried on by Mr. Simanton; in the second one by Mr. Plank; and in the third one by Mr. Fiske. All of the laboratory experiments were performed by Dr. McIndoo.

Lovett (5) reports that nicotine sulphate with soap is an effective ovicide for the codling moth. Excepting this experiment, in which only 26 codling-moth eggs were used, no other experiments, either in orchards or in a laboratory, have ever been conducted to determine how nicotine sulphate checks the work of the codling moth, although Feytaud (3), testing the effects of pure nicotine and soap in the laboratory upon the eggs of two moths belonging to the same family, ascertained that about 75 per cent of the embryos in the eggs tested were aborted during the last stage of development, while the remaining eggs hatched.

The first portion of the present paper embodies the results obtained in the laboratory concerning the effects of nicotine sulphate as an ovicide and larvicide on the codling moth and three other insects. The second portion of this study deals with the effectiveness of nicotine sulphate as compared with the efficiency of arsenate of lead for controlling the codling moth in orchards.

EXPERIMENTS CONDUCTED IN THE LABORATORY.

In order to throw some light on how nicotine sulphate checks the work of the codling moth the following tests were performed:

EFFECTS OF NICOTINE SULPHATE³ ON EGGS AND NEWLY HATCHED LARVÆ OF THE SILKWORM MOTH.

This investigation was begun in September, 1916, when it was too late to obtain codling-moth eggs or fresh eggs of any other insect; so the study was begun by using eggs of the silkworm moth (*Bombyx mori* L.), 10,000 of which were 14 months old, 2,000 of which were from 1 to 6 days old, and 10,000 of which were 3 months old.

EFFECTS OF NICOTINE SULPHATE ON EGGS OF THE SILKWORM MOTH.

The eggs just enumerated were divided into several lots, and some of these lots were sprayed with nicotine sulphate (1:800 + fish-oil

³ Throughout this paper reference is made to "nicotine sulphate" containing 40 per cent of nicotine.

soap, 2 pounds to 100 gallons of water) ; some with nicotine sulphate 1:800; some with nicotine sulphate 1:1,066; and others were not sprayed, these being used as controls. During October, 1916, all of the eggs 14 months old hatched, but the hatching of the sprayed ones was more or less retarded. Relative to the eggs from 1 to 6 days old when treated, the following data were obtained during the spring of 1917. Only 1 per cent of those sprayed with nicotine sulphate 1:800 hatched, while 80 per cent of the unsprayed ones hatched; in regard to the eggs 3 months old, 20 per cent of those sprayed with nicotine sulphate 1:800+soap hatched, 25 per cent of those sprayed with nicotine sulphate 1:800 hatched; 35 per cent of those sprayed with nicotine sulphate 1:1,066 hatched; while 90 per cent of those unsprayed hatched. The unsprayed eggs hatched quickly, and the newly hatched larvæ grew normally, while the sprayed eggs were more or less retarded in hatching and the young larvæ were so stunted that after 20 days the larvæ from the unsprayed eggs were from 1 to 6 times as large as those from the sprayed eggs. A large percentage of the unhatched eggs contained embryos aborted during the last stage of development.

EFFECTS OF NICOTINE ON NEWLY HATCHED SILKWORMS.

McIndoo (6, p. 97) has already shown that the exhalation (called odor) from leaves an hour after having been sprayed with a solution of nicotine sulphate kills small fall webworms (*Hyphantria cunea* Dru.), small caterpillars of the catalpa sphinx (*Ceratomia catalpae* Bdv.), and certain aphids (*Aphis populifoliae* Davis).

On May 12 at 12.30 p. m. a bunch of leaves on a mulberry tree was dipped into a solution of nicotine sulphate 1:400 and another bunch into a solution 1:800. On May 14 at 9.30 a. m. a leaf was removed from each bunch of the leaves treated, and 20 silkworms 1 day old were placed on each leaf; at 12.30 p. m. all the worms were dead. The leaves still emitted a faint odor like that from the solution. On May 16 at 11.15 a. m. the preceding experiment was repeated; on May 17 at 9 a. m. 8 worms on the leaf treated with the solution 1:400 and 1 on the other leaf were dead; all of the remaining live worms were more or less stupid, and two hours later, after having been fed with a fresh leaf treated with the solution 1:400, died. This leaf was cut into small pieces, and it still emitted a very faint odor like that from the solution, but the leaves treated with the solution 1:800 had ceased to emit this odor so far as the observer could perceive. At no time during any of the preceding experiments did the silkworms eat the treated leaves; the exhalation arising from them therefore must have killed the worms.

EFFECTS OF NICOTINE SULPHATE ON EGGS AND NEWLY HATCHED LARVÆ OF THE CODLING MOTH.

In tests with the eggs and newly hatched larvæ of the silkworm moth it was determined that nicotine sulphate is efficient against only the fresh eggs, but more or less retards the hatching of older eggs. The exhalation from leaves which had been dipped into solutions of nicotine sulphate from 1 to 5 days previously killed newly hatched silkworms, and from the sixth to the eleventh day after the foliage had been treated the newly hatched silkworms, after having eaten some of these treated leaves, died, the mortality varying from a high percentage on the sixth day to no mortality on the tenth and eleventh days. The following pages give the results obtained by using the eggs and newly hatched larvæ of the codling moth:

EFFECTS OF NICOTINE SULPHATE ON EGGS OF THE CODLING MOTH IN THE LABORATORY.

Many codling-moth sticks containing wintering larvæ, reared at the bureau's field station in Grand Junction, Colo., were shipped to Washington, D. C., where they were kept out of doors until March 1, 1917; after this date they were kept in a refrigerator, and at irregular periods about 40 of them at a time were removed from the refrigerator and were placed in battery jars, which were covered with cheesecloth. Each morning the moths that had emerged were removed to other battery jars containing moist sand and either glass plates or foliage of fruit trees. In these jars the moths copulated and the females laid their eggs on the glass plates and foliage, which after being removed were then sprayed. By this method eggs of a known age were easily obtained.

Since these experiments were started in March, when fruit-tree foliage was not to be had, glass plates were put in the battery jars, and whenever a sufficient number of eggs had been deposited on these plates, the latter were removed, sprayed, and then placed in other battery jars also containing moist sand.

Since the details of the four experiments performed, using eggs on glass plates, are similar, only Experiment No. 1 will be described fully. On April 4 the first plate, bearing 57 eggs (1 and 2 days old), was sprayed with a solution of nicotine sulphate 1:400; the second plate, bearing 20 eggs, with a solution 1:800; and a third plate, bearing 16 eggs, was used as a control. Each plate was put in a separate battery jar. On April 7 most of the eggs were in the red-ring stage; the sprayed plates bore spots or a thin film as a residue left after the evaporation of the solution, and these spots still emitted a nicotine-like odor. On April 11 many of the eggs were in the black-spot stage and the plates still emitted the nicotine-like odor; two small last year's apples were put in each jar. On

April 12 the eggs on each plate began to hatch, and later when examined 42.1 per cent of those on the first plate had hatched; 60 per cent of those on the second plate and 92.7 per cent of those not sprayed had hatched. On April 13 several dead larvæ were found on the sprayed plates and on the apples by these plates, but no dead ones were found in the jar containing the control plate, and four burrows were counted in the apple by this plate. On later dates it was common to see dead larvæ in each of the first two jars. On April 23 the plate sprayed with the solution 1:400 still emitted a faint nicotine-like odor. The apples by this plate showed four burrows, from which finally emerged two full-grown larvæ; those by the other sprayed plate only one burrow, from which emerged a larva; and those by the control plate also four burrows, from which emerged two large larvæ. Four of the five larvæ passed through the pupal stage and emerged as adult moths, while the fifth one only reached the pupal stage; this one was from the first two apples mentioned.

The preceding tests indicate that nicotine sulphate is not an efficient ovicide against the codling moth when the eggs are laid on glass plates. Other experiments were performed by constantly keeping fresh foliage of pear and apple trees in the battery jars. On the third day after applying the spray solution a nicotine-like odor was still emitted from the leaves, the strength of which depended upon the strength of the solution used. This odor gradually disappeared, and by the time the eggs began to hatch the observer could not perceive it. Unsprayed leaves usually have bright and shiny surfaces, but sprayed ones have dull surfaces, and with the aid of a hand lens a dried film of residue from the solution may often be seen on them. These experiments are summarized in Table I; experiments 1, 2, and 3 represent individual tests with eggs on pear-tree leaves, while experiment 4 is a summary of several tests with eggs on apple-tree leaves. Soap was used at the rate of 2 pounds per 100 gallons of water.

Relative to experiment 4, the following notes, although not significant, are nevertheless interesting. Four last year's apples, each being dipped into the same spray solution as that sprayed on the leaves, were placed in four jars containing the sprayed leaves. Two apples not sprayed were also placed in two jars containing unsprayed leaves. Eliminating one of the four jars and its contents on account of the apple in it becoming badly decayed before the eggs hatched, 245 eggs in the other three jars hatched and later 14 burrows were counted in the three remaining apples. In the two jars used as controls 52 eggs hatched, and later 14 burrows were also counted in the two apples contained therein. Soon after this date all of these apples totally decayed and consequently no matured larvæ were obtained from them.

Three apples, after having been dipped into a solution of nicotine sulphate 1:400+soap, were placed in three battery jars containing many unsprayed eggs. A later examination showed that 49 eggs had been deposited on the apples, 47 of which had hatched. These decayed apples contained 39 burrows, and finally two moths were found in each of two jars. A repetition of this experiment resulted in obtaining no moths from three dipped apples and only two from three control apples.

TABLE I.—*Effects of nicotine sulphate on codling-moth eggs (1 and 2 days old) laid on fruit-tree foliage in laboratory.*

Experiment No.	Strength of nicotine-sulphate solution used, and control.	Number of eggs sprayed.	Eggs hatched.	Embryos aborted.	Eggs totally undeveloped.
			<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1.	1:400.....	15	53.3	46.7	
	1:800.....	33	66.7	33.3	
	1:800+soap.....	15	63.7		36.3
	(Control.....	29	96.6		3.4
2.	1:1,000+soap.....	38	92.1	7.9	
	(Control.....	75	98.7		1.3
3.	1:800.....	52	63.5	13.4	23.1
	(Control.....	64	95.3	3.1	1.6
4.	1:400.....	116	47.4	52.6	
	1:400+soap.....	158	49.2	47.4	3.4
	1:800+soap.....	213	79.8	20.2	
	(Control.....	56	94.4		5.6

To test the value of nicotine sulphate as an ovicide for codling-moth eggs, two experiments were started by E. H. Siegler, entomological assistant, June 5, 1918, at the bureau's field laboratory in Wallingford, Conn. In each experiment the eggs were deposited on apple-tree foliage by moths confined in oviposition jars. All of the treated eggs were thoroughly immersed in a solution of nicotine sulphate 1:800 plus common laundry soap (3 pounds to 50 gallons of water). After treatment each leaf was placed in a separate jar, with an apple as food for the larvæ as they hatched.

Experiment No. 1.—In this experiment the eggs were deposited June 3, and at the time of dipping them into the spray solution many had advanced to the red-ring stage. Seven leaves bearing 310 eggs were treated, while one leaf bearing 190 eggs was left untreated as a check. The results obtained show that out of 310 eggs treated, 296, or 95.48 per cent, hatched; out of 190 untreated eggs 96.84 per cent hatched. Many of the larvæ entered the apples and commenced feeding vigorously therein.

Experiment No. 2.—The eggs employed in this test were deposited June 4, and at the time of dipping them into the nicotine-soap solution none had advanced to the red-ring stage. Out of a total of 37 eggs, 35, or 94.59 per cent, hatched; out of 11 untreated eggs, 11, or

100 per cent, hatched. As in experiment No. 1, many of the newly hatched larvæ entered the apples and started their development therein.

EFFECTS OF NICOTINE SULPHATE ON NEWLY HATCHED LARVÆ OF THE CODLING MOTH IN THE LABORATORY.

From the preceding results it is evident that nicotine sulphate is not an efficient ovicide against the codling moth; and since the eggs probably hatched after the cessation of an injurious exhalation from the sprayed glass plate and leaves, the newly hatched larvæ perhaps died of starvation, rather than from the spraying. To test the effects of the exhalation from leaves sprayed one or more days previously upon the newly hatched larvæ, the following experiments were performed:

At 11.30 a. m. a pear-tree leaf was dipped into a solution of nicotine sulphate 1: 400, and another one into a solution 1: 800. At 1.30 p. m., when the leaves were dry, 15 newly hatched codling-moth larvæ were placed upon each leaf. At 4.30 p. m. 13 larvæ were dead on the first leaf, but only 6 dead on the second leaf. The leaves still emitted a nicotine-like odor. The following morning all the larvæ were dead and the leaves were wilted. At no time did any of them try to burrow into the leaves as do larvæ on leaves not treated with these solutions, and they crawled away from these leaves more than do larvæ on untreated leaves.

On May 4 a twig bearing foliage on an apple tree was dipped into a solution of nicotine sulphate 1: 400, and another twig into a solution of 1: 800. On May 14 a leaf was removed from each twig and then 28 newly hatched codling-moth larvæ were placed upon each leaf between two watch glasses. During the first day the larvæ burrowed considerably in the leaves and apparently were not affected. During the second day three of them died, but the leaves began to wilt. During the third day all of them died, and by this time the leaves had become dry. Between May 4 and 14 there had been much rain and on the latter date the leaves did not emit a nicotine-like odor. This experiment shows that the exhalation from the treated leaves did not affect the larvæ and that in all probability all of them finally died of starvation.

On May 18 the two twigs mentioned in the foregoing paragraph were again dipped into solutions of the same strengths, but this time soap was added at the rate of 2 pounds per 100 gallons of water. On May 19 six newly hatched larvæ were placed on each of two detached leaves as usual. The leaves still emitted a faint odor resembling that from the solutions. No larvæ died that day and only 4 of the 12 were found dead the following day. The leaves were slightly eaten.

On May 20, at 1 p. m., or the second day after the leaves had been treated, 13 codling-moth larvæ were placed on each of two detached leaves; at 3 o'clock 6 silkworms (2 days old) were also placed on each leaf; at 4.30 o'clock 10 of the 12 silkworms were dead, but not one of the codling-moth larvæ had succumbed. The following morning all of the silkworms were dead, but only 12 of the 26 codling-moth larvæ had succumbed. The leaves were slightly eaten.

On the third day after the leaves had been treated the preceding experiments were repeated. On this date only a few of the silkworms and none of the codling-moth larvæ died. The following morning all of the silkworms were dead, but the codling-moth larvæ did not die till later, when the leaves began to dry.

On the tenth day after the leaves had been treated the codling-moth larvæ tested apparently were not affected.

On various dates many small pears (22 mm. in diameter) with the calyx cups yet open were picked and then sprayed with solutions of nicotine sulphate. Some of these solutions contained soap in the proportion of 2 pounds to 100 gallons of water. The stem of each pear used was inserted into a small bottle containing water. From one to seven days after the pears had been sprayed six active codling-moth larvæ were removed from the oviposition jars and then placed on each pear; unsprayed pears were used as controls.

A glance at Table II shows that about 75 per cent of the larvæ tested were killed when placed upon pears which had been sprayed one or two days previously. It is also seen that the mortality decreases to about 25 per cent on the sixth and seventh days.

TABLE II.—*Effects on newly hatched codling-moth larvæ when placed upon small pears previously sprayed with solutions of nicotine sulphate in laboratory.*

Number of days after spraying pears when larvæ were tested.	Solution 1:400		Solution 1:800.		Solution 1:400+soap.		Solution 1:800+soap.		Control.	
	Number of larvæ tested.	Larvæ found dead on pears.	Number of larvæ tested.	Larvæ found dead on pears.	Number of larvæ tested.	Larvæ found dead on pears.	Number of larvæ tested.	Larvæ found dead on pears.	Number of larvæ tested.	Larvæ found dead on pears.
		Per cent.		Per cent.		Per cent.		Per cent.		Per cent.
1.....	18	72.2	18	77.7	18	61.1	12	25.0	18	0.0
2.....	18	77.7	18	72.2	6	100.0	6	66.6	6	16.6
3.....	6	50.0	6	50.0	6	50.0	6	33.3		
4.....	6	83.3	6	66.6	6	33.3			6	16.6
5.....	12	25.0	6	33.3						
6.....	6	33.3	6	33.3						
7.....										

EFFECTS OF NICOTINE SULPHATE ON EGGS OF TWO OTHER INSECTS.

Experiments similar to those performed on the eggs of silkworm moths and codling moths were also conducted upon the eggs of

potato beetles (*Leptinotarsa decemlineata* L.) and tussock moths (*Hemerocampa leucostigma* S. & A.).

Fifteen potato-plant leaves, each bearing a cluster of potato-beetle eggs (1 and 2 days old), were collected in a potato patch, and after the petiole of each leaf had been inserted into a bottle of water in the laboratory, 11 of the leaves were sprayed with solutions of nicotine sulphate 1:400 and 1:800 and the other four leaves were used as controls. The eggs thereafter were observed daily; practically all of them hatched, and the newly hatched larvæ were apparently not affected by remaining on the wilted leaves 1 or 2 days, after which they thrived when fed fresh and unsprayed leaves. Relative to the time of hatching there seemed to be little, if any, difference between the sprayed and unsprayed eggs. Similar results were obtained by spraying eight clusters of potato-beetle eggs in a potato patch with a solution 1:400 containing soap (2 pounds to 100 gallons water).

In order to obtain tussock-moth eggs of known ages, many large caterpillars of this moth were collected and were put in wire-screen cages, in which the remainder of the life history was completed. On various dates eggs from these cages and some from trees in the field were collected, placed in open pasteboard boxes, and then sprayed with four solutions of nicotine sulphate. Two of the solutions contained soap in the proportion of 2 pounds per 100 gallons of water. Some of the control eggs were not sprayed, while the others were sprayed with tap water; no difference in hatching between these was observed. The details of these experiments are summarized in Table III:

TABLE III.—*Effects of nicotine sulphate on eggs of tussock moth in laboratory.*

Strength of nicotine-sulphate solution used, and control.	Eggs collected in cages.						Eggs collected in field. Age probably from freshly-laid eggs to those ready to hatch.		
	Eggs 1 and 2 days old.			Eggs 2 and 3 days old.			Number of egg masses tested.	Egg masses that hatched.	Egg masses that did not hatch.
	Number of egg masses tested.	Egg masses that hatched.	Egg masses that did not hatch.	Number of egg masses tested.	Egg masses that hatched.	Egg masses that did not hatch.			
		Per cent.	Per cent.		Per cent.	Per cent.		Per cent.	Per cent.
1:400.....				4	25.0	75.0	12	83.3	16.7
1:800.....				4	62.5	37.5			
1:800+soap.....	4	25.0	75.0	4	25.0	75.0	33	87.9	12.1
1:1000+soap.....	4	37.5	62.5				45	82.2	17.8
Control.....	3	83.4	16.6	4	87.5	12.5	45	93.3	6.7

It is seen from Table III that nicotine sulphate is not efficient against the eggs of the tussock moth; the percentages given for the masses of eggs that did not hatch, however, are too low, because the final examination showed that as a rule when a control and a sprayed

mass of eggs (both recorded as hatched) were closely observed, more larvæ had emerged from the former than from the latter. The ones sprayed with nicotine sulphate were slightly retarded in hatching, and the eggs in most of the masses which did not hatch contained aborted embryos, while the remainder of them were totally undeveloped.

DISCUSSION AS TO HOW NICOTINE SULPHATE ACTS AS AN OVICIDE AND LARVICIDE.

It is difficult to determine accurately how any insecticide kills a larva or an adult insect, and in the light of our present knowledge along this line it would perhaps be impossible to ascertain definitely how an ovicide affects insect eggs. For this reason no attempt was made in the present investigation to determine how nicotine sulphate killed the eggs tested. Nevertheless a little theory on this subject may not be out of place here.

Before attempting to explain how nicotine sulphate acts as an ovicide, it is first necessary to know something about the coverings of an insect egg. It is well known, as compiled by Packard (8, *p.* 520), that a ripe insect egg has two coverings. The external one, the egg-shell or chorion, is usually thick and hard; it is pierced by one or more minute canals, the micropyles, and consists of two chitinous-like laminae kept in close apposition by means of numerous minute pillars. The internal covering, the vitelline membrane, is always thin and delicate. The developing embryo inside the egg is supposed to breathe directly through these coverings as does a chick embryo through the shell of a hen's egg.

According to the results already presented, it was ascertained that freshly laid eggs, sprayed with solutions of nicotine sulphate, were affected more than were older eggs likewise treated. The chorions of these eggs were not as hard as those of the older eggs. This fact alone may explain the difference in the mortality recorded. Since it is scarcely possible that the spray solutions passed through the eggshells, there remain two possible methods of explaining the death of the eggs sprayed: (1) Upon evaporation of the spray solutions there was usually left a thin film on the leaves sprayed, and perhaps also on the eggs. This film on the eggs might have decreased the aeration through the eggshells and thus caused death by suffocation. (2) Since practically all of the aborted embryos appeared to have died during the last stage of development, the preceding view does not seem to have much weight. As the sprayed surfaces continued to emit a nicotine-like odor for some time after the spray solutions had been applied, it seems reasonable to suppose that the embryos were killed by breathing the exhalation from the sprayed surfaces through the eggshells. Nicotine is a nerve poison, and the more highly developed

the nervous system the more effective the poison. This fact probably explains why practically all of the embryos that died succumbed during the last stage of development.

Feytaud (3) described five stages in the embryology of (*Eudemis*) *Polychrosis botrana* Schiff. and of (*Phalonia*) *Conchylis ambiguella* Hbn., and he treated many eggs of these moths with various strengths of pure nicotine and soap. The resulting mortality varied considerably, but even the strongest solution (1:666) used did not prove efficient. He tested the eggs in the various stages of development, but in almost every case in which the embryos died they succumbed during the last stage.

Lovett (4) sprayed apple-tree foliage with solutions of nicotine sulphate, and after several hours, when the leaves were perfectly dry, he placed many small tent caterpillars (*Malacosoma pluvialis* Stretch) on these leaves. He states that these caterpillars showed a decided aversion for the sprayed leaves; all of them soon became sick and a large percentage of them died. On the third day after the foliage had been sprayed more caterpillars were placed on the same leaves and similar results were obtained. The leaves were not eaten. Lovett performed other experiments in which the caterpillars slightly ate the leaves which had been sprayed several hours previously. In this case the action of the nicotine was quick and decisive. These results agree with those given in the preceding pages.

Theoretically nicotine sulphate is nonvolatile, although we know from experience that the commercial 40 per cent nicotine sulphate (the kind used in all the preceding experiments) is more or less volatile. Moore and Graham (7) state that pure nicotine sulphate is nonvolatile, and demonstrated that its vapor or exhalation (if either one is given off) did not kill the insects tested. They also state that commercial 40 per cent nicotine sulphate contains from 1 to 2 per cent of free nicotine, whose vapor did kill the insects tested. Moore and Graham claim that when spray solutions are prepared by using commercial nicotine sulphate, hard water, and soap, the nicotine sulphate is decomposed, setting free the nicotine; the amount of decomposition depends upon the amount of alkalies contained in the solutions. This view easily explains the presence of a thin film (already mentioned several times) left upon evaporation of the spray solution, which is certainly the nonvolatile portion of the nicotine sulphate contained in the solution. For some time after the application of the solution the film continues to emit an odor and its exhalation is more or less toxic to young larvæ, showing that it is not pure nicotine sulphate according to Moore and Graham.

Moore and Graham (7) state that 12 days after lettuce in a greenhouse had been sprayed with a solution of commercial 40 per cent nicotine sulphate, it still bore nicotine.

To determine how long nicotine sulphate may remain on fruit-tree foliage after the application of the spray solution, the following experiments were performed by McIndoo. On September 10 a small pear tree was sprayed with a solution of commercial 40 per cent nicotine sulphate 1:800, containing fish-oil soap (2 pounds to 100 gallons water). On various days thereafter about a quart of the sprayed leaves were collected on each recorded date; the leaves, after being ground in a meat grinder, were then mixed thoroughly with water containing sodium carbonate. Upon distilling this mixture a distillate containing nicotine was obtained on each day tested up to the thirty-fifth day after the leaves had been sprayed. On the forty-second day after applying the spray solution only an indication of a precipitate was observed, but concluding from the precipitates obtained prior to this date the amounts of nicotine present in the distillates seemed to decrease irregularly from the first to the forty-second day; soon after which the remaining leaves fell from the trees. During these tests there was comparatively little rainfall; nevertheless the results of the tests indicate that considerable rainfall is required to remove all of the nicotine sulphate adhering to the sprayed leaves. The details of these tests are presented in Table IV:

TABLE IV.—*Tests on various dates using pear-tree leaves which had been sprayed on Sept. 10 with a solution of commercial 40 per cent nicotine sulphate (1:800) containing soap to show the presence of nicotine.*

Date.	Number of days after leaves had been sprayed.	Kind of precipitate obtained using—		Amount of rainfall.	Remarks.
		Phosphomolybdic acid.	Silicotungstic acid.		
Sept. 11	1	Heavy....	Heavy....	<i>Inches.</i> 0.00	Sprayed leaves emit nicotinlike odor.
Sept. 12	2	do.....	do.....	.00	Do.
Sept. 12	(¹) 3	None.....	None.....	.00	Unsprayed leaves on another tree were used.
Sept. 13	3	Heavy....	Heavy....	.00	Sprayed leaves do not emit nicotinlike odor.
Sept. 14	4	do.....	do.....	.14	
Sept. 15	5			.01	
Sept. 16	6			.08	
Sept. 18	8	Fair.....	Faint.....	.00	
Sept. 24	14	do.....	Very faint.	T.	
Sept. 27	17			.07	
Sept. 28	18			.16	
Oct. 1	21	Fair.....	Very faint.	.00	
Oct. 4	24			.01	
Oct. 5	25			.20	
Oct. 8	28	Fair.....	None.....	.07	
Oct. 9	29			.35	
Oct. 11	31			.01	
Oct. 12	32			.36	
Oct. 15	35	Very faint.	None.....	T.	
Oct. 19	39			.89	
Oct. 20	40			.01	
Oct. 22	42	Indication.	None.....		
Total amount of rainfall during tests...				2.36	

¹ Control.

At the suggestion of Mr. V. I. Safro, McIndoo washed separately 50 sprayed pear-tree leaves in 50 cc. of water. The resulting filtrate was acidified with hydrochloric acid and then tested for the presence of nicotine by adding phosphomolybdic acid and silicotungstic acid. Each test apparently showed the presence of nicotine. Fifty unsprayed leaves were likewise tested as a control; no precipitate was observed. One hundred unsprayed leaves were also tested, using the same amount of water; this time a precipitate was observed, and the same result was obtained thereafter on several occasions. Only green and unbroken leaves were used in these tests. At all times there is more or less foreign organic matter in the form of dust on the leaves; this is readily seen in microscopical sections through unsprayed leaves, and alkaloidal reagents precipitate it, as well as the nicotine which may be present. Consequently the preceding method should not be regarded as reliable; nevertheless, Mr. Safro (9) has published his method and says:

Generally we are able to obtain a definite indication of the presence of nicotine in as little as 25 cc. of water by using five leaves that had been sprayed at the usual strength (about 0.05 of 1 per cent nicotine).

On the fourth day after the pear tree used in the experiments had been sprayed, about a quart of the sprayed leaves were collected and washed in water containing sodium carbonate, each leaf being washed separately. Next the leaves were washed in running water for 5 minutes, and were finally ground and tested as described above. By adding phosphomolybdic acid to the resulting distillate, a faint precipitate was obtained, but none by adding silicotungstic acid. On the eighth day after the leaves had been sprayed, the test was repeated, but this time the leaves were washed for 10 minutes in running water. No precipitate was observed in this test even by adding phosphomolybdic acid. In each of the two foregoing tests the water containing the sodium carbonate and foreign matter removed from the leaves was distilled. Adding either acid to the resulting distillate invariably produced a precipitate, but in none of the tests with sprayed leaves did the distillate ever emit a nicotine odor. The second experiment described above indicates either that the spray solution did not pass into the leaves or that the amount of nicotine still held by them was too small to be detected by the method employed. The following results will substantiate the former view:

A small quantity of nicotine-sulphate solution (1:800) containing fish-oil soap (2 pounds to 100 gallons of water) was colored with carmine acid (Grübler's Carminsaure) and an equal quantity with indigo carmine (sodium sulphindigotate). Several leaves on a pear tree were sprayed with each of the colored solutions, and two entire detached leaves were submerged in each solution for 15 minutes; the

petioles of the leaves were left projecting above the surfaces of the solution. An hour later the four treated leaves were put into absolute alcohol and a day later a few of the stained leaves on the tree were likewise treated. The absolute alcohol did not dissolve or scatter either one of the stains, and the only other reagents used were xylol and Canada balsam; these also did not scatter the stains. A study of the sections made shows much stain on the outside of the sprayed and submerged leaves. While none was observed inside the sprayed leaves, it was usually present inside the submerged ones and the most of it seems to have passed through the ventral surfaces of the leaves. Its passage may have been aided by the stomata which occur exclusively on the ventral surface of these leaves; botanists, however, say that the stomata are so small (0.0006 mm. and less) that neither dust nor water can pass through them into the plant. According to the above results the spray solutions did not pass into the leaves sprayed, nor did the dust in the film of adhering nicotine sulphate enter the stomata.

SUMMARY OF EXPERIMENTS CONDUCTED IN LABORATORY.

Nicotine sulphate as an ovicide, with one exception, was found inefficient against all of the eggs tested—namely, those of the silkworm moth, codling moth, tussock moth, and potato beetle. The eggs sprayed with it were variously affected, depending on the strength of the spray solution used, on the age of the eggs tested, and whether or not the solution contained soap. Upon the eggs of three of the species of insects used there was practically no difference in effects between solutions containing soap and those without soap, although those with soap were much more effective upon the eggs of the tussock moth. Comparing the effects of the spray solution 1:800, the strongest one of the economic solutions used, the percentages of eggs sprayed with it that failed to hatch are as follows: Ninety-nine per cent of the freshly laid eggs of the silkworm moth, but only about 75 per cent of the older eggs of the same insect; about 20 per cent of the codling-moth eggs on apple-tree foliage; 75 per cent of the tussock-moth eggs (1 to 3 days old); and about 12 per cent of those of the same insect which had been collected in the field; and practically none of the potato-beetle eggs.

The effects of the exhalation from leaves sprayed with solutions of nicotine sulphate varied considerably, depending on the larvæ tested and the strength of the solutions used. Again comparing the effects of the solution 1:800, all of the newly hatched silkworms and codling-moth larvæ placed upon leaves sprayed two hours previously died, but the silkworms succumbed the more quickly; all of the silkworms but only a small percentage of the codling-moth larvæ placed upon leaves sprayed 24 hours previously died.

Seventy-seven per cent of the newly hatched codling-moth larvæ placed upon pears sprayed 24 hours previously with a solution of nicotine sulphate 1:800 died, but only 33 per cent of those placed upon pears sprayed a week previously died.

Relative to the control of the codling moth, the following briefly summarizes the more important points herein recorded: About 20 per cent of the freshly laid eggs sprayed with a solution of nicotine sulphate 1:800 failed to hatch, and one-third of the remaining embryos that emerged died before they entered pears sprayed a week previously. Hence about one-half of the eggs and larvæ had been destroyed up to the time when the remaining larvæ entered the sprayed pears; a large percentage of the latter larvæ died, as about 60 per cent of the pears did not become wormy. According to these laboratory results nicotine sulphate does not seem to be efficient against the codling moth, although only one application of it was made; nevertheless its effectiveness would certainly be increased in proportion to the number of its applications and to the amount of rainfall.

EXPERIMENTS CONDUCTED IN ORCHARDS.

According to the preceding laboratory results nicotine sulphate would not seem to be efficient against the codling moth. To determine this from the practical viewpoint the following experiments were conducted:

EXPERIMENTS PERFORMED AT BENTON HARBOR, MICH.

The experimental work conducted at Benton Harbor consisted in testing the relative efficiency of nicotine sulphate when combined with soap and when combined with soap and arsenate of lead in contrast with the efficiency of arsenate of lead when combined with lime-sulphur at summer strength. The experiments were made in a sod orchard about 50 years old, and each of the four plats selected consisted of 16 trees.

All of the applications throughout the season were made with the same power sprayer and at a pressure well above 100 pounds. The spray was delivered through the Vermorel type of nozzle. Although the experiments were started with the calyx application, all of the plats, including the check (No. 4), were given the cluster bud application.

The set of fruit was scattered and rather light, being lightest in plat No. 3, but it was sufficient to give reliable experimental results. In order that trees with as uniform a crop as possible might be secured throughout the different plats, five count trees in each plat were not selected until after the fruit had set. The fallen fruit was

collected once a week and examined for codling-moth injury, and at picking time the picked fruit was also examined. The results of these examinations are given in Table V.

TABLE V.—*Codling moth injury as shown by examination of both fallen and picked fruit from experimental plats at Benton Harbor, Mich., 1917.*

No. of plat.	Number of applications and brief formula of each.	Fruit record.				
		Number of larval entrances at—			Number of wormy apples.	Total number of apples examined.
		Calyx.	Side.	Stem.		
1	1 application of lime-sulphur and 3 applications of lime-sulphur + 1 pound arsenate of lead to 50 gallons water.....	191	277	14	336	6,543
2	1 application of lime-sulphur and 3 applications of nicotine sulphate 1:800+soap.....	559	662	71	1,249	9,924
3	1 application of lime-sulphur and 3 applications of nicotine sulphate 1:800+soap+0.5 pound of arsenate of lead to 50 gallons of water.....	40	195	4	231	2,949
4	1 application of lime-sulphur (check).....	2,032	607	93	2,484	6,060
						59.00

Reference to Table V shows that nicotine sulphate (plat No. 2) gave a control of only 87.41 per cent, while arsenate of lead (plat No. 1) gave a control of 94.71 per cent. There is thus seen to be no practical advantage in combining arsenate of lead with nicotine sulphate in sprays designed to control the codling moth (plats Nos. 1 and 3).

EXPERIMENTS PERFORMED AT GRAND JUNCTION, COLO.

Experiments similar to the preceding were also performed at Grand Junction, Colo. Of the 12 plats sprayed, only 2 were sprayed with nicotine sulphate, but since the results obtained using arsenate of lead vary so greatly, the data from 4 of the latter plats are given in Table VI, so that the results from the plat sprayed with nicotine sulphate may be compared with those from the plats sprayed with arsenate of lead alone and also with the results from the plat sprayed with arsenate of lead and nicotine sulphate combined. In plats on which it was applied (i. e., all except plats Nos. 10, 14, and 15), the calyx application was made with a Bordeaux type of nozzle which delivered a fairly coarse and driving spray; a mist type of nozzle was used for all subsequent applications. With this exception the same spraying equipment was used for all plats except the checks.

At various times throughout the growing season and at harvest time the apples on eight trees in each plat (four in each of the two check plats) were examined; the results of these examinations, together with the treatment of the several plats, are given in Table VI.

TABLE VI.—*Codling-moth injury as shown by examination of both fallen and picked fruit from experimental plats at Grand Junction, Colo., 1917.*

Plat No.	Formula of insecticide.	Number of applications.			Total number of apples examined.	Fallen apples free from worms.	Harvested apples free from worms.	Total apples free from worms.
		For first brood.	For second brood.	Total.				
						<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
2	1 pound of powdered arsenate of lead to 50 gallons of water.....	4	2	6	23,508	37.75	72.16	62.09
3	Nicotine sulphate (1:800).....	4	2	6	29,881	17.32	58.33	37.96
4	1 pound of powdered arsenate of lead to 50 gallons of water plus nicotine sulphate (1:800).....	4	2	6	24,003	40.97	84.17	67.50
8	1 pound of powdered arsenate of lead to 50 gallons of water.....	3	2	5	38,874	48.28	82.31	73.49
9	Same as above.....	4	3	7	47,773	47.74	85.53	76.53
10	Same as above.....	13	2	5	37,628	22.83	60.69	46.33
14 and 15	Check (unsprayed).....				33,882	7.36	15.01	8.72

¹ All three were cover applications, the calyx application being omitted.

Although the results in the preceding table vary considerably, it is seen nevertheless that nicotine sulphate when used alone was inefficient (plat No. 3).

EXPERIMENTS PERFORMED AT ROSWELL, N. MEX.

The experimental work conducted at Roswell consisted in a comparison of the efficiency of nicotine sulphate with that of arsenate of lead alone and also with that of arsenate of lead combined with nicotine sulphate. For this purpose four plats, lying in a 640-acre tract of apple trees, were selected; they were similar in all respects and the trees, about 20 years old, were of the Ben Davis variety. Following the regular 5-spray schedule, all the plats, except the check (No. 4), were sprayed on the following dates: April 25, May 15, June 14, July 18, and August 14. During the first application about 21 gallons of spray material were applied to each tree, but during each subsequent application only about 17 gallons per tree were used. Bordeaux nozzles were used for the calyx application and a mist type for the other applications. A power sprayer, holding 200 gallons, was employed, and the pressure was maintained at 200 pounds with three leads of hose.

During these experiments the dates and amounts of rainfall are as follows: May 6, 0.23 inch; July 19, 0.03 inch; July 20, 0.03 inch; August 1, 0.54 inch; August 2, 0.47 inch; August 15, 0.15 inch; August 17, 0.60 inch; and August 18, 0.93 inch, giving a total of 2.98 inches.

The details pertaining to the plats, formulas of application, and results of the foregoing experiments are given in Table VII.

TABLE VII.—*Codling-moth injury as shown by examination of both fallen and picked fruit from experimental plats at Roswell, N. Mex., 1917.*

Plat No.	Number of applications and formula of each.	Fruit record.			
		Total number of apples examined.	Fallen apples free from worms.	Harvested apples free from worms.	Total apples free from worms.
1	5 applications of 1 pound of powdered arsenate of lead to 50 gallons of water.....	1 33,472	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
2	5 applications of 1 pound of powdered arsenate of lead to 50 gallons of water+nicotine sulphate (1:800)+ $\frac{3}{4}$ pound of laundry soap.....		93.64	97.66	96.88
3	5 applications of nicotine sulphate (1:800)+ $\frac{3}{4}$ pound of laundry soap to 50 gallons of water.....	2 7,011	85.55	98.99	97.00
4	Unsprayed.....	3 6,170	88.01	93.43	91.94
		4 14,401	52.06	50.01	51.53
1 On 6 trees.		2 On 4 trees.		3 On 4 trees.	
				4 On 12 trees.	

From Table VII it is seen that the trees sprayed with nicotine sulphate (plat No. 3) yielded a crop of apples 92 per cent sound, while those sprayed with arsenate of lead (plats Nos. 1 and 2) yielded a crop 97 per cent sound, and that the addition of nicotine sulphate to the arsenate of lead mixture (plat No. 2) did not materially increase the percentage of sound fruit.

CONCLUSIONS FROM FIELD EXPERIMENTS.

According to the work conducted during the season of 1917, it is shown that nicotine sulphate 1:800 with soap gave a fair degree of control for the codling moth at Benton Harbor, Mich., and at Roswell, N. Mex., but that it was not as effective as 1 pound of powdered arsenate of lead to 50 gallons of water; and also that there was no practical advantage in combining arsenate of lead and nicotine sulphate in sprays designed to control the codling moth. Of course it is well known that nicotine sulphate controls aphids on fruit trees, but this phase of the subject is not considered in this paper. At Grand Junction, Colo., where the infestation was much heavier, nicotine sulphate 1:800 without soap was inefficient against the codling moth.

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