UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

Research Bulletin 202

Are We Developing Strains of Codling Moths Resistant to Arsenic?

LEONARD HASEMAN and R. L. MEFFERT

(Publication Authorized July 24. 1933)

COLUMBIA, MISSOURI

AUGUST, 1933

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Are We Developing Strains of Codling Moths Resistant to Arsenic?

LEONARD HASEMAN and R. L. MEFFERT

When an insect pest for any reason ceases to be effectively controlled by a particular insecticidal treatment, there is a tendency for the entomologist to jump at the conclusion that the insect has developed a resistance to that insecticide. Such conclusions appear more logical when a particular strain of an insect pest more successfully withstands an insecticide treatment than some other strain of the same insect when both strains are subjected to identical conditions. Also, the fact that man and higher animals can actually develop an increased tolerance for certain poisons adds weight to the theory that insects may become more resistant to insecticides. In this case, however, the individual develops the resistance by repeated exposure to increased dosages, while we would be obliged to assume that only those individual insects which had developed a tendency to resist the insecticide would survive and in turn would transmit that tendency to future generations.

It seems evident that if a particular species of insect has developed an increased resistance, or is developing such resistance to a poison, it should be able to consume more of the poison and survive than it was able to do years ago. Also, if a particular strain of a species of insect shows greater resistance than other strains, then the resistant strain should be able to consume a larger dose of the poison than the less resistant strains. Some years ago before the present epidemc of the codling moth began to build up, the senior author began an investigation of the dosage of arsenic lethal to codling moth larvae and the best methods of maintaining such a dosage on the fruit and foliage where the larvae would get it. In these earlier investigations measured doses of arsenicals were fed to the larvae in small pits made in the apple. The results submitted in this report were secured by injecting measured doses of arsenicals directly into the digestive tract and into the haemocoele. In order to eliminate any possible variation which the digestive secretions of the different strains might have on the arsenical and any variations which their digestive tracts might show as regards either absorption or expulsion of the arsenic, we studied the effect of injections both into the digestive tract and directly into the body cavity.

REVIEW OF LITERATURE

Investigators in the medical profession have devoted considerable time to a study of the action of arsenic on man and higher animals, and there are a number of valuable papers dealing with

*The data presented in this bulletin were taken from a thesis submitted by the junior author in fulfillment of the thesis requirement for the degree of Master of Arts in the Graduate School of the University of Missouri, 1932.

the action of arsenic on insects and the dosage lethal to different insects, but very little work has been done on the possible development of resistance to arsenic by insects.

Meyer and Gottliebe[1]* say that repeated and careful administration of arsenic by the stomach leads to an increase in tolerance, so that quantities three or four times the ordinary fatal dosage can be borne without ill effects.

According to Voegtlin, Dyer and Leonard[2], in higher animals arsenic may be considered as a specific poison for reduced glutathione or the toxic action of arsenic may be due to an interference with the normal function of glutathione in oxidation and reduction phenomena of the tissue.

Fink[3] found that insects subjected to arsenical treatment showed marked reduction in glutathione content, presumably indicating that glutathione is an arsenical receptor. In an earlier investigation Fink[4] utilized the oxygen consumption depression as a basis for judging the relative toxicity of different arsenicals.

Tartar and Wilson[5] using the common tent caterpillar found by chemical analysis of dry tissue of dead larvae that acid arsenate of lead acted much more quickly and effectively than did basic arsenate of lead.

Lovett and Robinson[6] using the same methods and working with the same insect found that only a portion of the arsenic eaten by the caterpillar was absorbed, the rest being passed off with the excreta.

Campbell[7] states that susceptibility of silkworm larvae decreases during larval development.

Moore[8] made chemical analyses of red-legged grasshoppers which had been fed a mixture of poison bran mash and found that more than 50 per cent of the arsenic was passed out in the excreta. He also found that sodium arsenite was twenty-eight times more toxic than arsenate of lead.

McIndoo and Cook[9] in determining the dosage of metallic arsenate lethal to various insects found that arsenites were much more toxic than arsenates.

Campbell[10] using a needle-pointed micro-burette for determining the dosage of arsenic lethal to silkworms found that the toxic action of arsenic depended on two factors, velocity of chemical action leading to death and the rate of distribution, excretion and penetration of arsenic. Arsenic by subcutaneous injection showed much more speed of action than through the alimentary canal.

Haseman[11] in determining the dosage of arsenic lethal to codling moth larvae fed known doses in pits made in apples and found that a dose of 0.0005 grams of arsenate of lead proved fatal to second and third instar larvae.

*Numerals refer to Bibliography, Page 11.

For a number of years there has been a feeling among entomologists that insects are developing a resistance to insecticides. In 1908 Woglum[12] stated that at that time it required twice as much hydrocyanic acid gas to give good control of the California red scale as it did when the gas was first used on the scale in that section.

Melander[13] in 1914 noticed an increase in resistance of San Jose scale to the lime sulphur solution.

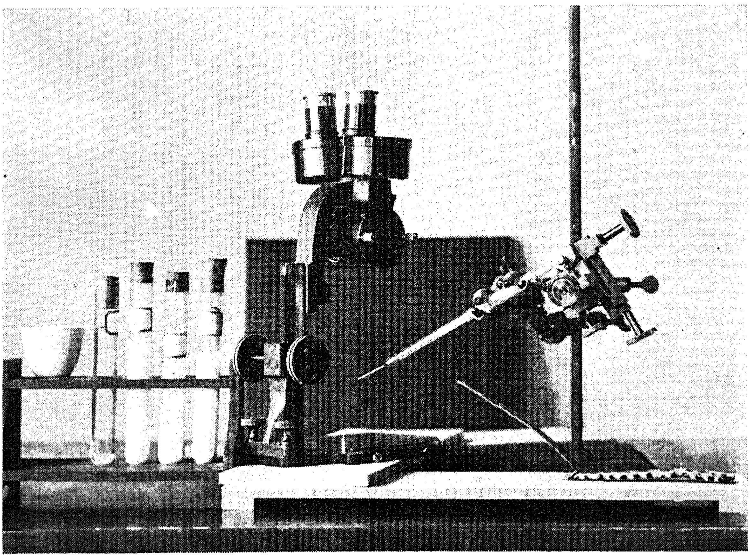
Hough[14] in studying the relative resistance of Colorado and Virginia codling moth larvae to arsenical sprays found that the former were markedly superior to the latter when it came to numbers of worms which succeeded in entering the fruit without being poisoned. Also this quality was transmitted to their offspring. The progeny from crossing the two strains were less successful in entering the fruit than were their Colorado parents but more successful than the Virginia parent and for, at least, two generations this held true. He thought that it might be due to a development of resistance to arsenic by the Colorado strain.

Haseman and Burk[15] by using the apple pit method of feeding measured dosages of arsenic to codling moth larvae of Missouri and Colorado strains found that the larvae from Colorado were killed with the same doses of arsenic required to kill Missouri larvae of similar weight. They concluded, therefore, that the Colorado strain had not developed resistance to arsenic.

PLAN OF PROCEDURE

It was our purpose in this investigation, first, to develop, if possible, a technique for administering very small but accurately determined doses and, second, to find out if the Colorado strain of codling moth larvae was able to withstand larger injections of arsenic than the Virginia or Missouri strains. A small hypodermic syringe with an extension graduated to .005 of a cubic centimeter was used for measuring and administering the doses. The syringe as shown in the illustration, was firmly clamped to an improvised stand so that the tip of the needle could be held directly beneath the objectives of a binocular microscope. The plunger of the syringe was manipulated by means of a micro-screw so that doses as small as .005 cubic centimeter could be administered with precision.

The investigation was carried on during the winter of 1931-32. Two thousand codling moth larvae were collected in Central Missouri during the fall of 1931 for use in the experiment, and during January, 1932, consignments of hibernating larvae were received from Professor G. M. List of Fort Collins, Colorado and from Dr. W. S. Hough of Blacksburg, Virginia. The larvae were



Apparatus used in measuring and administering the doses.

kept in hibernation under outdoor conditions, and as needed a few were taken into the laboratory and kept under room temperature for a day before using them. By using mature hibernating larvae and by selecting them according to weight, we felt that they would all be in as nearly identical condition for testing their susceptibility to arsenic as we could hope to get them.

In these experiments both sodium arsenite and acid arsenate of lead were used. Distilled water was used for preparing series of dilute solutions of the two poisons so that, when injections of .005 cc. or .01 cc. of the different solutions were made, known dosages were administered. Injections were made both through the mouth into the digestive tract and directly into the body cavity. As checks, distilled water in equal quantities was injected into control specimens. When the injections were carefully made the mechanical injury proved to be of no consequence. However, in all cases where there was any bleeding, or where the larvae died in ten minutes after the injection was made, or where for any reason the larvae failed to get the full dose of the poison they were discarded.

In administering the dose through the mouth the larvae were held between the thumb and forefinger and, with the aid of the binoculars, the tip of the needle was inserted in the mouth and the proper quantity of liquid injected by turning the micro-screw to force in the plunger. When the injections were made into the body cavity the same plan was followed, the needle being inserted just above the spiracles in the region of the sixth body segment.

In perfecting the technique and in working out the approximate lethal dose for the mature worms large numbers of Missouri larvae were used. The larvae were treated in groups of from 20 to 30. They were then placed in corrugated paper and examined at the end of four- and eight-hour intervals. In comparing the susceptibility of the Virginia, Missouri and Colorado larvae the same plan was followed.

LETHAL DOSAGE OF SODIUM ARSENITE IN THE HAEMOCOELE

The approximate lethal dosage was determined by injecting groups of ten Missouri larvae with known doses of sodium arsenite. If all died in the first four hours, the dose was reduced one-half and given to another group of ten larvae. If all were alive at the end of four hours the dose was doubled and used on another group of larvae. In this way the approximated dosage was readily determined. Then four stock solutions were prepared so that when .005 cubic centimeter of the different solutions was used to inject into the larvae the amounts administered were .00000125, .0000020, .0000025 and .00000275 gram of sodium arsenite. Table 1 shows the result where these solutions were injected into the haemocoele.

TABLE 1.—SUBINTEGMENTAL INJECTION OF SODIUM ARSENITE. VOLUME OF DOSAGE IS .005 OF A CUBIC CENTIMETER.

No. of Larvae	Date	Dosage Gm. of Sodium Arsenite	Number Killed 4 Hrs.	Number Killed 8 Hrs.	Number Alive 4 Hrs.	Number Alive 8 Hrs.	Percentage Killed 4 Hrs.	Percentage Killed 8 Hrs.	Remarks
25	1/ 2/32	0.00000125	8	--	17	--	32	--	
20	1/ 3/32	0.00000125	6	10	14	10	30	50	
20	1/ 3/32	0.00000200	8	9	12	11	40	45	
30	1/ 5/32	0.00000225	18	22	12	8	60	70	
20	1/ 6/32	0.00000250	12	14	8	6	60	60	
30	1/ 9/32	0.00000250	24	--	6	--	80	--	
30	1/15/32	0.00000250	17	20	13	10	56	66	
50	1/19/32	0.00000275	46	50	4	0	92	100 -	This is the approximate minimum lethal dosage.
50	1/20/32	0.00000275	42	47	8	3	84	94	
CHECK									
15	1/ 2/32	Distilled H_2O	0	0	15	15	0	0	
20	1/ 5/32	Distilled H_2O	0	0	20	20	0	0	
20	1/ 7/32	Distilled H_2O	2	2	18	18	10	10	

LETHAL DOSAGE OF SODIUM ARSENITE IN THE ALIMENTARY CANAL

We should naturally expect that a larger dose of arsenic would be required to kill larvae where it is injected into the alimentary canal than where it is injected directly into the body cavity or blood stream. Seven stock solutions were prepared so that the weakest one contained the same concentration as the strongest solution used in the previous experiments. When .005 cubic centimeters of these stock solutions were injected into the digestive tract, the larvae received the following doses of sodium arsenite: .00000275, .0000030, 00000325, 00000350, .00000375, .0000040, and .00000425 gram. Table 2 shows the results where these doses were injected into the digestive tract.

TABLE 2.—DOSAGE OF SODIUM ARSENITE IN THE ALIMENTARY TRACT. VOLUME OF DOSAGE IS .005 OF A CUBIC CENTIMETER.

No. of Larvae	Date	Dosage	Number Killed		Number Alive		Percentage Killed		Remarks
		Gm. of Sodium Arsenite	4 Hrs.	8 Hrs.	4 Hrs.	8 Hrs.	4 Hrs.	8 Hrs.	
16	2/8/32	0.00000275	2	2	14	14	12.5	12.5	
12	2/8/32	0.00000275	3	--	9	--	25	--	
24	2/10/32	0.00000300	8	10	16	14	33	42	
20	2/10/32	0.00000300	2	9	18	11	10	45	
30	2/10/32	0.00000325	12	14	18	16	40	46	
20	2/12/32	0.00000325	8	8	12	12	40	40	
22	2/12/32	0.00000350	14	14	8	8	63	63	
20	2/13/32	0.00000350	12	--	8	--	60	--	
30	2/14/32	0.00000375	20	20	10	10	66	66	
20	2/16/32	0.00000375	14	--	6	--	70	--	
20	2/20/32	0.00000400	14	15	6	5	70	75	
30	2/20/32	0.00000400	23	24	7	6	76	80	
20	2/21/32	0.00000400	14	--	6	--	70	--	
20	2/23/32	0.00000425	16	16	4	4	80	80 -	This is the approximate
30	2/23/32	0.00000425	23	24	7	6	76	80	minimum lethal dosage.
CHECK									
20	2/18/32	Distilled H_2O	0	0	20	20	0	0	
20	2/20/32	Distilled H_2O	0	0	20	20	0	0	
30	2/21/32	Distilled H_2O	0	0	30	30	0	0	

Tables 1 and 2 indicate that the minimum lethal dose of sodium arsenite for mature Missouri codling moth larvae is .00000275 gram when injected directly into the body cavity and .00000425 when administered through the alimentary canal, showing that 54% more is required to kill when applied in the digestive tract.

LETHAL DOSAGE OF ACID ARSENATE OF LEAD IN THE ALIMENTARY CANAL

As in the case of sodium arsenite, a series of trial injections were made to determine the sublethal dose and then three stock solutions were prepared so that when injections of .01 cubic centimeter of the solutions were made the larvae received doses of 0.00019, 0.00024 and 0.00048 gram of arsenate of lead. In the case of arsenate of lead the lethal dose was determined only when administered in the alimentary tract. Table 3 shows the results when acid arsenate of lead was injected into the alimentary tract. When the results shown in Tables 2 and 3 are compared, it is found that sodium arsenite is about one hundred and thirteen times as active as acid arsenate of lead.

TABLE 3.—THE DOSAGE OF ACID ARSENATE OF LEAD IN THE ALIMENTARY TRACT. VOLUME OF DOSAGE IS .01 OF A CUBIC CENTIMETER.

No. of Larvae	Date	Dosage Gm. of Arsenate of Lead	Number Killed 4 Hrs.	Number Killed 8 Hrs.	Number Alive 4 Hrs.	Number Alive 8 Hrs.	Percentage Killed 4 Hrs.	Percentage Killed 8 Hrs.	Remarks
30	3/2/32	0.00019	6	--	24	--	20	--	
30	3/3/32	0.00019	4	--	26	--	13	--	
33	3/3/32	0.00024	10	21	20	12	33	63	Arsenate of lead acts more slowly than sodium arsenite
30	3/4/32	0.00024	8	19	20	12	35	63	
15	3/4/32	0.00024	4	11	11	4	26	73	
20	3/4/32	0.00048	14	17	6	3	70	85	
15	3/8/32	0.00048	10	13	5	2	63	85	

(Note: The same check was used in this experiment as was used in Experiment 2.)

RELATIVE RESISTANCE OF MISSOURI, COLORADO AND VIRGINIA STRAINS OF CODLING MOTH LARVAE TO SODIUM ARSENITE

In testing the relative susceptibility of the three strains of larvae a sublethal dose of 0.00000375 gram of sodium arsenite was used to inject into the alimentary canal. The same technique was used as in determining the lethal dose. Table 4 shows the results. From these results it is evident that there is no marked difference in the three strains as regards susceptibility to arsenic.

TABLE 4.—EFFECT OF SODIUM ARSENITE ON STRAINS OF CODLING MOTH. VOLUME OF DOSAGE IS .005 OF A CUBIC CENTIMETER.

No. of Larvae	Date	Dosage Gm. of Sodium Arsenite	Number Killed 4 Hrs.	Number Killed 8 Hrs.	Number Alive 4 Hrs.	Number Alive 8 Hrs.	Percentage Killed 4 Hrs.	Percentage Killed 8 Hrs.
		MISSOURI STRAIN						
30	2/15/32	0.00000375	20	20	10	10	66	66
30	2/17/32	0.00000375	18	--	12	--	68	--
20	2/19/32	0.00000375	12	14	8	8	60	70
20	2/20/32	0.00000375	14	14	6	6	70	70
20	2/23/32	0.00000375	13	--	7	--	65	--
30	2/27/32	0.00000375	18	20	12	10	60	66
		COLORADO STRAIN						
30	2/15/32	0.00000375	18	19	12	11	60	63
30	2/17/32	0.00000375	19	--	11	--	63	--
20	2/19/32	0.00000375	13	--	7	--	65	--
24	2/20/32	0.00000375	14	15	10	9	58	62
20	2/23/32	0.00000375	12	--	8	--	60	--
30	2/27/32	0.00000375	20	--	10	--	66	--
		VIRGINIA STRAIN						
30	2/15/32	0.00000375	14	16	16	14	46	53
30	2/17/32	0.00000375	15	16	15	14	50	53
20	2/19/32	0.00000375	10	11	10	9	50	55
20	2/20/32	0.00000375	12	13	8	7	60	65
20	2/23/32	0.00000375	11	--	9	--	55	--
30	2/27/32	0.00000375	18	--	12	--	60	--

(Note: The weight of 20 Missouri larvae is .7228 gm. The weight of 20 Colorado larvae is .6980 gm. The weight of 20 Virginia larvae is .7314 gm. All of the strains were approximately the same size and in the same stage of development.)

RELATIVE RESISTANCE OF MISSOURI, COLORADO AND VIRGINIA STRAINS OF CODLING MOTH LARVAE TO ACID ARSENATE OF LEAD

The sublethal dosage of 0.00024 of a gram of acid arsenate of lead was used to inject into the digestive tract of the three strains of larvae. The same technique as in earlier tests was used and Table 5 shows the results. These records show that the Colorado strain was more susceptible to arsenate of lead, if anything, than were either the Virginia or Missouri larvae.

TABLE 5.—EFFECT OF ACID ARSENATE OF LEAD ON STRAINS OF CODLING MOTH. VOLUME OF DOSAGE IS .01 OF A CUBIC CENTIMETER.

No. of Larvae	Date	Dosage	Number Killed		Number Alive		Percentage Killed	
		Gm. of Arsenate of Lead	4 Hrs.	8 Hrs.	4 Hrs.	8 Hrs.	4 Hrs.	8 Hrs.
		MISSOURI STRAIN						
28	3/ 5/32	0.00024	9	18	19	10	32	64
30	3/ 7/32	0.00024	12	17	18	13	40	60
33	3/11/32	0.00024	16	21	17	12	48	63
	3/17/32	0.00024						
		COLORADO STRAIN						
26	3/ 5/32	0.00024	14	20	12	6	53	76
45	3/ 7/32	0.00024	24	39	21	9	53	86
20	3/11/32	0.00024	--	15	--	5	--	75
15	3/17/32	0.00024	--	10	--	5	--	66
		VIRGINIA STRAIN						
30	3/ 5/32	0.00024	12	20	18	10	40	66
33	3/ 7/32	0.00024	--	21	--	12	--	63
25	3/11/32	0.00024	--	16	--	9	--	64
18	3/17/32	0.00024	--	11	--	7	--	62

(Note: All the larvae were approximately the same weight and in the same stage of development.)

SUMMARY

(1) A dosage of 0.00000275 gram of sodium arsenite when injected into the haemocoele of fullgrown codling moth larvae gives a kill of from 85 to 90 per cent within four hours.

(2) A dosage of 0.00000425 gram sodium arsenite when injected through the mouth into the digestive tract of fullgrown codling moth larvae gives a kill of from 75 to 80 per cent within four hours.

(3) Sodium Arsenite is a little more than one and one-half times as toxic when injected into the haemocoele than when introduced into the alimentary canal.

(4) A dosage of 0.00024 gram acid arsenate of lead when introduced through the mouth into the alimentary canal gives a kill of from 26 to 33 per cent in four hours and from 63 to 73 per cent in eight hours.

(5) Sodium arsenite when injected into the alimentary canal is about one hundred and thirteen times as toxic to fullgrown codling moth larvae as is acid arsenate of lead and it kills in one-half the time.

(6) The sublethal dosage of 0.00000375 gram of sodium arsenite when injected into the alimentary canal gives approximately the same percentage of kill of the Colorado, Missouri and Virginia strains of fullgrown codling moth larvae. The Colorado larvae shows no greater resistance to total water soluble sodium arsenite than do the Missouri and Virginia larvae.

(7) When injected through the mouth into the alimentary canal the sublethal dosage of 0.00024 gram of acid arsenate of lead gives approximately the same percentage of kill of the Colorado, Missouri and Virginia strains of fullgrown codling moth larvae.

(8) The fact, therefore, that it is more difficult to control the Colorado strain of codling moth larvae with arsenical sprays than is the case with Missouri and Virginia larvae is evidently not due to their having developed a resistance to arsenic.

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