Arsenates: Effect on Fecundity in Some Diptera

Abstract. *Four species of adult Diptera,* Rhagoletis pomonella *(Walsh),* Drosophila melanogaster *Mg.,* Drosophila hydei *Sturt., and* Musca domestica *L. showed varying degrees of reduced fecundity when sublethal doses of arsenic were included in their food. The possible influence of the frequent use of arsenicals on arthropod populations is discussed.*

The use of chemosterilants (*1*) for the control of insect pests and the anomalous results frequently recorded in the use of arsenates for control of the apple maggot, *Rhagoletis pomonella* (Walsh), suggested the possibility that arsenates might influence fecundity. When larvae of *Bupalus piniarius* L. consumed sublethal doses of arsenical dusts on pine trees the pupae were noticeably smaller and the fertility of the females was impaired (*2*). Nenyukov and Tareeva (*3*) suggested that incomplete poisoning (with arsenicals) affects metabolic processes in insects and would probably reduce their reproductive power. Their test insects were cockroaches and *Dendrolinus sibiricus* Tshtv.

Laboratory tests during 1962 on four species of Diptera, *Rhagoletis pomonella, Drosophila melanogaster* Mg., *D. hydei* Sturt., and *Musca domestica* L. showed that egg production was greatly reduced when the newly emerged adults were fed sublethal doses of arsenates. It is not clear whether or not the interference is of a permanent nature, but the initial suppression of ovarian development and egg formation is very marked in the first three species, though less so in *M. domestica.* In the tests on *R. pomonella,* the arsenates of lead or calcium mixed with the food (soy hydrolyzate and honey) at concentrations from 0.5 to 0.1 percent reduced egg development by 80 percent or more. As the flies emerged they were placed in lantern-globe cages and were provided with a constant supply of food and water. Flies that died within 14 days were excluded from the count. At concentrations of lead arsenate from 0 to 0.1 percent there were no great differences in fly longevity although, on the average, the flies lived somewhat longer in the check cages. The ovaries were examined under a stereoscopic microscope about 3 weeks after emergence and divided into three groups, according to the number of eggs, as follows: normal, reduced (five or less eggs per ovary), and nil (no eggs showing). The number of flies in each group for the different treatments are shown in Table 1.

Cages were placed on small potted apple seedlings that had been dipped in aqueous suspensions of lead arsenate at concentrations of 0.1 and 0.05 percent; food and water were provided in the cages so that it would be unnecessary for the flies either to feed or to rest on the dipped plants. Under these conditions *Rhagoletis pomonella* produced fewer eggs than they did when the arsenates were mixed with the food. This is probably explained by the fact that in nature the flies appear to search continually for food, and this characteristic persists in the laboratory cages where the flies were almost continuously active on the plants, seemingly searching for or imbibing food. They fed on the artificial food rather infrequently and apparently supplemented their diet from this source only when the amount of food taken from the plants was inadequate. The results from these tests are shown in Table 2.

Lead arsenate had no adverse effect on the fertility of the male flies.

An orchard test confirmed the laboratory results. A heavily infested, well isolated orchard, was sprayed with lead arsenate, at a concentration of 0.3 percent, on 17 July 1962, just as the earliest flies emerged in the orchard. Oviposition was prevented for over a month. Only in the last week in August did a substantial percentage of the flies show mature eggs in the ovaries. Flies taken from this orchard in September showed scarcely more than half as many eggs in the ovaries as those taken from an untreated orchard. Flies which emerged in the orchard and were placed in cages on unsprayed fruit before they fed developed their eggs normally and laid many eggs; similar flies caged on sprayed limbs developed very few eggs, even though many of them lived for 4 weeks or more. The flies in all cages were supplied with food and water and hence were not obliged to gather food from the foliage or fruit. A comparison of soil samples taken from beneath the trees in November 1961 and 1962 indicated a reduction in puparia of 98 percent in the one year from the lone application of lead arsenate. Failure of arsenical sprays to provide adequate control of this pest in orchards nearer sources of infection is attributed to invasions of flies that have not fed to an appreciable extent on foliage or fruit treated with arsenicals and, therefore, have their eggs well developed at the time of arrival in the treated orchard. It is known that the flies will travel several hundred yards, especially with the wind, but the maximum distance has not yet been determined.

Table 1. Number of *R. pomonella* with egg complements of various sizes when food contained the indicated amounts of the arsenates.

Arsenate (%)	Normal	Reduced	Nil
	Control		
0	83	6	11
	Lead arsenate		
0.5	0	0	10
.1	7	38	93
.05	21	27	22
	Calcium arsenate		
.1	4	8	22

Although the compounds tested (arsenates of lead, calcium, and sodium) appeared to produce similar effects—they repressed ovarian development—it has not yet been determined whether the effect is permanent or only temporary. There was an indication that *Rhagoletis pomonella* flies, when fed arsenates in their diet for 3 days immediately after emergence from the puparium and then fed a diet without arsenates, were slower in developing their eggs than those that received no arsenates; however, some of the flies eventually developed what appeared to be full complements of eggs. As a consequence of these observations we are uncertain whether or not these arsenates fit Borkovec's (*1*) definition of a chemosterilant as "a chemical compound which, when administered to an insect, will deprive it of its ability to reproduce." Thus the compounds seem to depress egg development so long as the fly continues to obtain small quan-

Table 2. Number of *R. pomonella* with egg complements of various sizes when arsenates were placed on apple seedlings.

Arsenate (%)	Normal	Reduced	Nil
	Controls		
0	16	1	0
	Lead arsenate		
0.1	4	17	47
.05	14	19	22
	*Calcium arsenate**		
.1	2	7	14

* In bordeaux mixture.

tities of the active ingredient; if this administration is continued for a considerable period of time the fly becomes sterile. Up to a certain point, however, the fly retains its inherent ability to produce eggs, provided it is supplied with suitable food.

Some interesting speculations arise from these results. The fact that these arsenates suppressed egg production in all four species of Diptera selected for laboratory testing because they were readily available, suggests to us that arsenates may influence the fecundity of many species, or other orders of insects, or even other classes of animals.

The question may then be raised whether arsenicals have influenced the establishment and development of parasites and predators in orchards during the many years that these chemicals were used in very large quantities. Because we have found that four species of Diptera, the only ones tested, are influenced in this way, it seems possible that parasitic and predaceous Diptera may be similarly affected.

H. T. Stultz of this laboratory studied the parasites of the eye-spotted bud moth, *Spilonota ocellana* (D. and S.), for many years and observed on numerous occasions that the braconid *Agathis laticintus* (Cresson), although almost absent from orchards sprayed regularly with arsenicals, would frequently appear in substantial numbers on trees so treated if there were unsprayed apple trees nearby. The parasite developed to the adult stage in the sprayed trees but never increased in numbers until the use of arsenicals was discontinued. Unfortunately no examination of the ovaries of these braconids was made and there is no proof that they were not killed directly by the arsenates; however, Stultz has suggested that the tissues of the larvae of *S. ocellana* may have contained sufficient arsenic to affect reproduction in *A. laticintus*.

These tests emphasize the necessity for determining more exactly the effects of pesticides on the pests against which they are directed; a mere counting of living or dead, or of survivors, is not enough. They suggest also the desirability of considering more precisely the effects of the applied pesticides on the faunal complex as well as on the higher animals which may come in contact with the chemical (*4*).

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References and Notes

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5 March 1963

Gibberellin: Effect on Diffusible Auxin in Fruit Development

Abstract. *Diffusible auxin is not present in tomato flowers at anthesis, but significant amounts can be obtained after the plants are treated with gibberellin. The early growth of the ovary in plants treated with gibberellin corresponds closely with growth after pollination and fertilization; the amounts of diffusible auxin are not significantly different over a period of 22 days.*

One of the more remarkable effects of gibberellin in plant growth is the induction of parthenocarpy by smaller quantities than those required for the hormone, indole-3-acetic acid (IAA) to produce the same effect. In tomatoes, parthenocarpy is induced by as little as $10^{-5}M$ gibberellic acid (GA) in lanolin, while $10^{-2}M$ IAA is the minimum effective application. Treatments with 10 μl of $3 \times 10^{-4}M$ solutions of nine gibberellins cause a significant increase in the diameter of tomato fruits (*1*). Several investigators have suggested that gibberellin may be a principal factor along with IAA in the control of fruit development (*2*).

Although there are reports of an increase in extractable auxin in plant tissues after treatment with gibberellin (*3*), one investigation showed no change in diffusible auxin after treatment (*4*). Treatment with GA causes a doubling or tripling of diffusible auxin in stem apices of peas and a tenfold increase in stem apices of sunflowers corresponding with the increased growth of the plants (*5*). Since the stimulus of pollination and fertilization also causes an increase in diffusible auxin in the ovary (*6*), we have examined the effect of treatment with GA on diffusible auxin in ovary tissue of the tomato.

Flowers of the Waltham Forcing variety of tomato (*Lycopersicon esculentum* L.) were emasculated at anthesis and 1 day later were treated with GA (85 percent, in lanolin at concentrations of $3 \times 10^{-4}M$ and $3 \times 10^{-3}M$. At intervals of 28 and 51 hours the flowers were cut at the base of the calyx and placed on blocks of 1.5 percent agar, 2 by 2 by 2 mm, in a moist chamber under 100 lu/ft^2 of cool white fluorescent light. Diffusion of auxin into the agar blocks took place during a period of 2 hours. The auxin content of the agar blocks was then measured by the standard Avena curvature test (*7*). The IAA concentration equivalent to the curvature was determined from curvatures induced by $2 \times 10^{-7}M$, $6 \times 10^{-7}M$ and $2 \times 10^{-6}M$ concentra- of IAA.

Four experiments gave essentially the same results. Although diffusible auxin is present during early stages of flower development, at anthesis there is none; without pollination and fertilization the flower abscisses. Within 28 hours after treatment with $3 \times 10^{-4}M$ and $3 \times 10^{-3}M$ GA there is an equivalent IAA concentration of $2.7 \times 10^{-7}M$ and $3.2 \times 10^{-7}M$ respectively. Fifty-one hours after treatment with GA the equivalent IAA concentration is $4.6 \times 10^{-7}M$ for both treatments. No diffusible auxin was obtained from ovaries 28 and 51 hours after treatment at anthesis with plain lanolin. The amount of diffusible auxin in the ovary apparently does not depend on the concentration of GA applied.

The effect of GA treatment was also compared with the effect of pollination and fertilization by measuring the diffusible auxin in ovaries during several

Table 1. Diffusible auxin obtained from normally developing tomato fruits and ovaries treated with gibberellic acid. The values at 22 days were obtained by using an agar block 4 by 2 by 2 mm for diffusion and cutting it into 4 blocks 2 by 2 by 2 mm for assay.

Treatment	Diameter of ovary (mm)	Curvature (degrees)	Equivalent IAA concentration ($10^{-7}M$)
3 days after treatment			
Pollinated	4.1±0.4	6.2±1.4	3.4
$3 \times 10^{-4}M$	3.8±0.1	8.6±1.1	4.9
$3 \times 10^{-3}M$	4.1±0.3	8.7±1.2	5
6 days after treatment			
Pollinated	9.2±0.6	13.±0.7	7
$3 \times 10^{-4}M$	8.0±0.5	9.9±0.7	4.6
$3 \times 10^{-3}M$	9.7±0.4	11.9±0.9	6.2
15 days after treatment			
Pollinated	29.0±1.1	16.0±1.6	14
$3 \times 10^{-4}M$	26.7±1.9	16.6±1.5	15
$3 \times 10^{-3}M$	28.1±1.1	17.3±1.6	16
22 days after treatment			
Pollinated	28.3±2.0	14.0±1.2	41
$3 \times 10^{-3}M$	32.3±3.8	11.2±1.7	26