HOW DOES LEAD ARSENATE PREVENT THE YOUNG CODLING MOTH LARVA FROM INJURING THE FRUIT?[1]

A. D. Heriot

Dominion Entomological Laboratory, Vernon, B.C.

Introduction

Information on the behaviour of newly hatched codling moth larvae on foliage and fruit has been given in previous papers from the Dominion Entomological Laboratory at Vernon; Heriot and Waddell (1942), Waddell and Marshall (1942). The following account represents an extension of these investigations. The conclusions and inferences are based partly on investigations reported in the literature and partly on original work.

Laboratory experiments with lead arsenate against first instar codling moth larvae have not, as a rule, given the degree of control that is generally attained under orchard conditions, although even in the latter case results often leave much to be desired. Nevertheless, lead arsenate still holds a prominent position in the codling moth spray schedule and it continues to be used in the field as the standard of effectiveness for experimental larvicides.

Not many years ago, it was sufficient to explain any apparent ineffectiveness of this material as being due to careless spraying. That explanation no longer suffices, since examples of poor control despite thorough application, are now commonplace in the arid or semi-arid apple-growing districts of Western North America. The reason for this lack in control, apart from the possible segregation of a race of codling moth resistant to insecticides, may become apparent when a clearer idea is obtained of how and when the action of lead arsenate takes place.

Results of Experiments, with Comments

The results of experiments bearing directly on the question under discussion, cover the period extending from the time the larva prepares to leave the egg until it enters the fruit. Where figures are given in this brief summary, experiments have been replicated many times. Where figures are omitted, the evidence is merely suggestive.

A heavy residue of lead arsenate on the chorion of the egg failed to prevent the larva from biting its way out during eclosion. This is not surprising as the chorion is generally torn open by the mandibles rather than bitten through, and to correspond with a residue on the fruit, the lead arsenate would have to be on the surface attacked, namely, the inner surface of the chorion.

After the larva leaves the egg, it must first locate the fruit and then find a suitable site for entry. On sprayed trees this generally entails crawling over a residue on the apple for 30 minutes to several hours. That this should afford ample opportunity for particles of arsenical to come in contact with the mouthparts and preoral cavity is indicated by the following experiment: 67 per cent of the larvae that crawled over a residue of finely divided graphite on Bristol board, picked up and sometimes swallowed particles of graphite in the space of 5 minutes, notwithstanding the fact that the surface was foreign to the normal environment of the larvae and presumably unattractive.

All larvae that crawled for one hour over the surface, showed particles of graphite in the ventriculus. On the other hand, when lead arsenate was added to the graphite, only 12 per cent instead of 100 per cent of the larvae were found to have graphite in the ventriculus; furthermore, less graphite was ingested by each larva. It is difficult to account for this difference if lead arsenate re-

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mained completely insoluble and hence inactive. It seems logical to believe that the arsenical became sufficiently soluble within the preoral cavity to be detected by the larva and that solubility was effected by the secretion of the mandibular glands ("saliva"). If lead arsenate in solution is repellent in the case of admixed graphite-lead arsenate, then it is conceivable that ingestion of graphite particles would be reduced and the lead arsenate, at least in particulate form, might be even more strongly rejected. As for solubilized arsenic, the amount inadvertently ingested would appear to be negligible, at least from the standpoint of direct lethal effect, since larvae may crawl over a lead arsenate residue for several hours without apparent injury.

It was found that larvae were much more reluctant to feed upon apple leaves that had been sprayed with lead arsenate than upon unsprayed leaves. Only 20 per cent of the larvae attacked sprayed leaves after being restricted to these for 15 hours, whereas under similar conditions, unsprayed leaves are readily fed upon. Ninety per cent of the larvae that actually fed on sprayed leaves lived for 15 hours or more although some consumed as much as 5 square millimeters of leaf. A similarly high percentage of larvae survived after successful entries through a residue of lead arsenate deposited over a puncture made in the skin of the apple with the point of a dissecting needle.

Experiments at this laboratory indicate that the repellence of lead arsenate residue may be so great that a high percentage of larvae may starve rather than bite through it, and further, when leaf tissue or apple pulp is consumed the repellence of lead arsenate is reduced. On the other hand, when sodium arsenite was substituted for lead arsenate on apples with the cuticle removed, few larvae fed and none entered. Perhaps the degree of acidity of the fruit and leaves is sufficient to maintain lead arsenate in an insoluble condition as it passes quickly into the mouth, while the more soluble sodium compound is not so affected.

Investigations show that larvae will consume particulate lead arsenate with food, and in the same way soluble arsenic may sometimes be ingested. In the case of larvae that fed upon apple pulp treated with sodium arsenite, there soon was a complete cessation of the peristaltic movements of the stomodaeum, although movement persisted for some time in the ventriculus and proctodaeum. When lead arsenate is ingested with food, peristaltic movement appears likewise to be affected though to a lesser degree. Figure 1 shows the normal distribution of food after 5 minutes of feeding on pulp dusted with graphite. Figure 2 shows the distribution of food with graphite plus lead arsenate after 2 hours. In the latter case, as in numbers of others under like treatment, clots of food were held up in the mouth, in the pharynx, and in the crop instead of normally passing on to the ventriculus.*

Voskresenskaya, whose work is discussed at some length by Hoskins (1940) records retention of food in the foregut, arising out of the use of sodium arsenite on three different insects, namely, the cutworm, the cabbage worm, and the cricket. She explains this as due to the depressing influence of arsenic on the nervous system governing intestinal movement. This produces a relaxation of the sphincter lying between the crop and midgut, presumably brought about by absorption of arsenic in the midgut. Figures 2, however, suggests a relaxation of the muscles of the pharynx governing swallowing, as well as a relaxation causing stoppage in the crop. This condition may be due to lead arsenate breaking down in the stomodaeal fluid. There appears to be no conclusive evidence that the walls of the stomodaeum are impermeable to substances in water solution. As a matter of fact, their derivation

* The crop does not appear to act as a receptacle for storage except when the insect has to be tided over a period of quiescence.

from the ectoderm and the demonstration that the ectoderm in wireworms is permeable to aqueous solutions of arsenic (Woodworth 1938) may be significant.

A symptom of what is regarded as stomach poisoning but which may be this condition of stoppage in the crop, is a clear watery exudation from the anus. A spot of the glistening residue of this excrement near the site of a larval injury is generally a sign that the larva responsible for it has died. Regardless however, of the precise manner in which death is brought about by the ingestion of lead arsenate, a most important point from the standpoint of the fruit-grower is that in the majority of cases death by ingestion apparently does not happen soon enough to prevent injury to the fruit.

Discussion

Acid lead arsenate is only slightly soluble in water and there is nothing to suggest that it affects the codling moth larva in the ordinary sense of a contact insecticide. Until the importance of the pH value of the digestive fluids was realized, it was difficult to understand how this very stable material could even act as a so-called stomach "poison". It is now generally assumed, somewhat too readily perhaps, that lead arsenate is ingested and breaks down to liberate soluble arsenic in the alkaline digestive fluids of the ventriculus, absorption then taking place and arsenic passing into the blood stream to pervade the tissues of the body with lethal effect.

This sequence of events which often requires a matter of hours or days, is known to occur in some leaf-eating lepidopterous larvae. These, when feeding on sprayed foliage, swallow the arsenical with their food. The amount of arsenic experimentally administered to such larvae has in some cases, been recovered from the excreta, blood, and tissues. (Voskresenskaya, as quoted by Hoskins 1940).

When restricted to a diet of lead arsenate-sprayed leaves, the young codling moth larva swallows the arsenical with its food. Incidentally, of course, the leaves are injured. But since in the orchard lead arsenate usually prevents the larva from injuring the fruit even by so much as a sting, it is reasonable to infer that the action of lead arsenate when on the fruit must be different in some respects from its action when on the leaves.

Possibly the situation is further complicated. Mature larvae, chiefly because of their size, have been generally used for direct experimentation with stomach insecticides. But as shown in figures 3 and 4 the young larva is not a small edition of later instars. Relatively it possesses a far greater proportion of nerve tissue. Indeed it might be aptly described as a "bundle of nerves" in contrast to the condition obtained in the mature larva. Is it too much to speculate that with such a profound change in anatomy there might go a change in physiology; that the first instar larva might be more susceptible either to the lethal effect or to the repellent effect of a compound such as lead arsenate?

If lead arsenate must pass into the ventriculus before it can be broken down, the time required for this and the subsequent chain of events leading to death, would apparently allow the larva to cause noticeable injury to the fruit. Under favourable conditions, an entry can be effected in 15 to 30 minutes, yet experiments indicate that when unaccompanied by food, ingested solids such as graphite, carmine, and lampblack require from 30 to 60 minutes to reach the ventriculus. Therefore, if lead arsenate is to prevent blemishes, it must exert action prior to arrival in the ventriculus.

It is common knowledge that preliminary to feeding, the larva removes and rejects the comparatively tough and dry cuticle of the fruit; actual feeding only taking place when the moist tissues of the cortex are exposed. In the task of penetrating the cuticle, the mouthparts of the larva are engaged in "spitting out" each fragment of cuticle laboriously chewed off by the mandibles. This procedure is therefore the very reverse of that accompanying ingestion. The removal of the cuticle is a slow process and the amount of effort expended in chewing off each fragment can be measured by the fact that this operation requires from 30 to 40 seconds for its accomplishment and has to be repeated 20 to 30 times to expose the pulp. During this time, an arsenical deposit on the cuticle would seem to have an excellent opportunity of encountering the epipharynx which is studded with spine-like projections deflected to the rear. With this equipment the labrum as shown in Figure 4, becomes an ideal instrument for removing and retaining a residue in the preoral cavity as each fragment is rejected. Under such circumstances, the deposit would be subjected to unobstructed action of a copious secretion issuing from the highly-developed glands which open at the base of the mandibles (Figure 5) and, as suggested from its alkalinity, this secretion should be capable of breaking down lead arsenate. Granting the presence of suitable chemoreceptors within the preoral cavity as delineated in Figure 6, the larva might come under the influence of soluble arsenic almost immediately an attempt was made to "sting" the fruit. Moreover, since it has been demonstrated by other workers that arsenicals may be distinctly repellent to a variety of insects, it seems reasonable to conclude that repellent action may indeed take place in the manner described. According to Ripley and Petty (1932), the greater its repellence the less the likelihood of the toxicant being ingested.

Hoskins (1940) reviews recent contributions of physiology to insect control. Practically all the experiments discussed are concerned with adult insects or well-developed larvae, and Hoskins points out that susceptibility to toxicants may vary according to age. He refers to the saliva as the first body fluid with which an ingested substance comes in contact, but goes on to state that "no data showing an effect of insect saliva upon the toxicity of an insecticide seem to have been recorded."

The only work cited by Hoskins in his wide survey that appears to have a direct bearing on this point is that of Marshall (1939). From colorimetric and potentiometric determinations of the pH values of the digestive fluids of the codling moth larva, Marshall gives the following results: neutral or slightly acid in the proctodaeum; approximately pH 8.5 in the ventriculus; and slightly more alkaline in the crop. A statement that regurgitated fluid had a pH value of 9 and over appears to be significant when consideration is given to the fact, that so far as is known, the stomodaeal fluid arises only by intermittent regurgitation from the ventriculus, and from the secretions of the mandibular glands. (The labial or true salivary glands in the codling moth larva are given over to the production of silk). If, then, the stomodeal fluid should be more alkaline than that in any other portion of the alimentary canal it is quite possible this condition is brought about by a strongly basic secretion functioning as saliva but emanating from the mandibular glands.

According to Woodworth (1938), in the case of the wireworm, repellency of an arsenical may be such that none is ingested even while the insect burrows into an arsenically-treated bait. The wireworm has no salivary or mandibular glands and Woodworth's statement that arsenicals are repellent in accordance with their solubility is of interest. Woodworth moreover, surmises that sensory control of the mechanism of the mouthparts may account for the rejection of arsenicals by the wireworm. The nature of the sensoria responsible for this is not stated. Dethier (1937) claims that caterpillars in general have a strong sense of taste which is said to reside in the preoral cavity. He identifies no specific gustatory organs.

There is the authority of Weber (1933) for the supposition that the sensilla coeloconica or pit-peg sensoria in the preoral cavity could serve as gustatory organs. How these organs are innervated does not seem to be clear. As far as can be determined from the literature, it would appear that in lepidopterous larvae, the seat of olfactory responses lies in the tritocerebrum and that of gustatory responses in the frontal ganglion (Figure 7). Sectioning of grown larvae by the writer did not reveal a connection between the frontal nerve and the epipharyngeal organs. The question arises as to whether sensoria may be innervated in the first instar larvae yet not in later instars. It is hard to conceive of the senses of taste and smell being unconnected with the stomodaeal nervous system.

It was demonstrated by McIndoo (1929) that certain responses to external stimuli are positive in the young codling moth larvae and negative in older larvae. This perhaps, is another way of saying that sense organs that are functional in the first instar may be non-functional at a later stage, a possibility indicated in Figure 6, where one pair of chemoreceptors of the final instar are vestigial and the other pair are situated some distance from the periphery of the epipharynx. Also to be borne in mind in this connection, is the super-abundance of nervous tissue in the first instar larva.

So far as has been determined, it is only when the larva is effecting an entry into the fruit that the fragments of sprayed cuticle are held in the preoral cavity for an appreciable length of time. When the larva rejects these fragments some of the residue may be retained in the preoral cavity and the "saliva" may thus have an opportunity to bring into solution a sufficient amount of arsenic to stimulate the sensoria of the epipharynx. As noted earlier, it is believed that these sensoria initiate an avoidance response.

Although repellent action may appear to explain how lead arsenate prevents the larva from injuring the fruit, it by no means follows that lead arsenate is never ingested without food or that when it is ingested with food, it does not injure the larva. If the "saliva" is capable of breaking down lead arsenate on the threshold of the mouth, it should be equally capable of acting as a solvent when lead arsenate is conducted into the foregut.

With regard to ingestion without feeding, Marshall (1937) draws attention to the aggregations of particles of lead arsenate picked up by the larva in wandering over a residue and suggests a possible relationship between particles adhering to the mouthparts, and ingestion. He demonstrates that these aggregates assume greater proportions on oily than on non-oily deposits. Experiments with inert dusts indicate that some of these accumulations adhering to the mouthparts may be forced into the buccal cavity when the larva attempts an entry. It is not known whether repellent particles are ingested in the same way or whether the larva is deterred from even attempting an entry under such circumstances. But apart from repellent or toxic action, Marshall, Strew and Groves (1939) have shown that a residue of zinc oxide, presumed inert, may exert a definite influence on control evidently by physical or mechanical means.

A concluding word regarding the apparent ineffectiveness of lead arsenate despite thorough application: In laboratory experiments with larvicides it has been customary to use 10 or more larvae per apple. Smith (1926) used as many as 25 larvae per apple. Recent research has suggested that the use of such numbers of larvae may lead to erroneous conclusions. One or more larvae may partially remove the cuticle with its residue before becoming impotent, and other larvae may then exploit these preformed sites of attack in safety, to effect entries. Repeated experiments have shown that approximately half the larvae individually applied to apples are able to find a single minute puncture made with the point of a dissecting needle, even when the larvae is deposited on the opposite side of the fruit to that on which the puncture is situated. Accordingly, it appears to be likely that in the orchard, larvae frequently and with success avail themselves of the attempted entries of predecessors. This may be a reason why inverted spray mixtures with their heavy deposits are better than non-inverted mixtures; why no residue other than one having a high contact value can be entirely effective; and why difficulty of control seems to increase more than proportionately as larval populations become greater.

Summary

Several ways are discussed in which a residue of lead arsenate may prevent young codling moth larvae from injuring fruit. Certain types of residue involving oils have been demonstrated to exert an influence on control by their physical or mechanical characteristics alone, but lead arsenate-casein-lime residue may owe its effectiveness chiefly to its repellent qualities.

Repellence is indicated by the reluctance of the larva to attack sprayed leaves and fruit; it appears to be at a maximum when the larva is removing the cuticle of the fruit prior to feeding. This task involves the "spitting out" and rejection of sprayed particles, a procedure which in part because of the structure of the labrum may result in some of the residue being retained in the preoral cavity. The strongly alkaline fluids present in the preoral cavity are believed to react quickly with acid lead arsenate and cause liberation of soluble arsenic. When soluble arsenic comes in contact with sensoria situated on the epipharynx, an avoidance response may be at once initiated.

Direct toxic action by ingestion seems to be the exception rather than the rule. Experiment suggests that it is doubtful if the various processes incident to systemic poisoning can take place with sufficient rapidity to prevent serious blemishes to the fruit.

The ordinary lead arsenate-casein-lime residue appears to become progressively less effective as a repellent as the larval population in an orchard becomes greater. This effect seems to result from the ability of the larvae to discover and to exploit previous attempted entries.

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