# **Alfalfa Caterpillar Tests**

biological control by artificial spread of virus disease studied

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Exploratory tests conducted in the summer of 1948 suggest the possible use of a virus as a means of biological control of the alfalfa caterpillar.

The results and conclusions of the tests are tentative but they do indicate that:

1. A virus, applied as a spray, is capable of causing infection in and markedly reducing populations of the alfalfa caterpillar.

2. An epizoötic-an epidemic among non-humans-of a disease can be started in low populations of 20 to 30 larvae per 10 sweeps, and that even these populations can be substantially reduced.

3. It is possible to initiate an epizoötic of the disease earlier than it would occur naturally, thus curtailing the amount of damage done the crop by the insect.

#### Tests

A virus disease-polyhedrosis-of the alfalfa caterpillar, Colias philodice eurytheme Bdvl., long has been known to be an important factor in the natural reduction of populations of this insect in California. These natural outbreaks of disease often occur so late in the larval life of the insect population that most of the damage to the crop has occurred by the time the caterpillars are destroyed by the virus.

These polyhedral virus diseases-commonly called wilt diseases-are characterized by the formation in the nuclei of the infected tissue of large numbers of microscopically visible inclusion bodies, called polyhedra, and usually by a marked liquefaction and distintegration of internal body tissues. The virus particles themselves, which are too small to be seen with an ordinary microscope, are for the most part contained within the polyhedra bodies.

As far as has been demonstrated, only the alfalfa caterpillar is susceptible to the polyhedral virus studied in these experiments.

The concentrated infectious material was taken into the field and then diluted so that the final spray solution was between 50,000,000 and 100,000,000 polyhedra per milliliter-a milliliter is about one twenty-ninth of an ounce. To apply the spray, an ordinary five-gallon backpack hand sprayer was used. The spray was directed uniformly over the alfalfa.

Four separate tests were conducted on plots situated in alfalfa fields in central California in the Westley-Patterson area of the northwest San Joaquin Valley. Comparable plots in the same fields were established as untreated controls. In all cases, the end of the experiment was dictated by the cutting of the field by the grower.

#### Plot No. 1

Test plot No. 1 and its control plot were 20 by 400 feet each. One gallon of virus suspension-97,000,000 polyhedra per milliliter of water-was applied to the alfalfa in this plot. The population of 58 larvae per 10 sweeps at the time of application of the virus rose to 154 per 10 sweeps in the control plot in 15 days. In the plot treated with virus suspension, the larval count remained parallel to that of the untreated plot during the incubation period of the disease-about six days-and then fell to four per 10 sweeps in 15 days.

That the mortality was caused by the virus is indicated by the fact that prac-



The number of alfalfa caterpillar larvae per 10 sweeps in the treated and untreated (control) portions of plot 1, during a 15-day period.

tically 100% of the larvae in the test plot was infected with the virus, as determined by laboratory examination, within four days after the virus was applied. By the end of the experiment, the incidence of infection in the control plot had risen to 82% because of the occurrence of a natural epizoötic.

The figures obtained indicate that a full-fledged epizoötic can be initiated artificially approximately 10 days prior to the time when a natural outbreak of the disease would occur.

#### Plot No. 2

Test plot No. 2 and control plot No. 2 were 25 by 360 feet each. The test plot was treated with 1.5 gallons of virus suspension-66,000,000 polyhedra per milliliter. This experiment was initiated on September 23 and closed on September 30 when the field was cut for hay. On the last day of the experiment no actively moving larvae were found in the test plot. Most of those collected in the sweepings were dead. In the control plot, al-



DATE (SEPTEMBER, 1948)

The number of alfalfa caterpillar larvae per 10 sweeps in the treated and untreated (control) portions of plot 2, during a 7-day period.

though there was a slight drop in the population, none of the larvae collected showed any evidence of the disease when examined in the laboratory.

The significance of this test lies in the fact that an epizoötic was established in such a small population, never greater than 38 larvae per 10 sweeps. The fact that no active larvae were found in the test plot after seven days probably does not represent true eradication of the pest in the plot, but it does indicate a very marked reduction in numbers.

#### Plot No. 3

Test plot No. 3 and its control plot were 75 by 300 feet each in size. One gallon of the virus suspension was applied at a concentration of approximately 57,000,000 polyhedra per milliliter. The population of 35 larvae per 10 sweeps dropped to eight per 10 sweeps as a result of the disease.

As in test plot No. 2, the significance of this test is in the indication that populations of relatively small densities can be reduced by the disease. Although this

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plot received the lowest concentration of virus for the area involved, it showed the sharpest reduction in the larval population.



#### DATE (AUGUST, 1948)

The number of alfalfa caterpillar larvae per 10 sweeps in the treated and untreated (control) portions of plot 3, during a 6-day period.

#### Plot No. 4

The size of the fourth test plot was 225 by 750 feet. The control plot of the same dimensions ran parallel to the test plot but about 100 feet from it. Fifteen gallons of virus suspension-50,000,000 polyhedra per milliliter-were applied to the alfalfa of the test plot.

This experiment was concerned with the largest area of any of the tests and the prolonged egg-laying by the adult butterflies produced young larvae which were coming on continuously, making the test a very severe one.



The number of alfalfa caterpillar larvae per 10 sweeps in the treated and untreated (control) portions of plot 4, during a 14-day period.

The number of larvae in the control plot rose from four per 10 sweeps to 353 per 10 sweeps in 14 days. In treated plots the population rose to 60 per 10 sweeps by the sixth day, then rose slightly until on the fourteenth day the count was 98 per 10 sweeps. During the incubation period of the disease the counts approximated each other.

The slight increase in numbers undoubtedly was caused by the great influx of newly hatched larvae, since at no time did the number of fourth- and fifth-instar larvae in the test plot exceed 20 per 10 sweeps.

One of the outstanding things indicated by this experiment is that in the fields treated with virus suspension, the caterpillar population was held below an economic level, whereas in the untreated control plot, the population rose to over 350, a level considerably higher than that at which the insect began to cause marked economic damage.

## Conclusions

The data obtained in these experiments do not, in themselves, constitute conclu-

sive proof that populations of the alfalfa caterpillar can be regularly controlled by the artificial distribution of the polyhedrosis virus. More extensive tests need to be completed before a safe conclusion can be drawn. Such tests are being planned.

In earlier tests it was found that the soil and surface debris of alfalfa fields naturally contain infectious virus material, indicating that the virus is probably widely distributed in nature. Apparently the new-grown alfalfa leaves do not have on them enough virus, or the virus is not dense enough to insure contact with early instar larvae.

Distributing the virus artificially by spraying increases the concentration of the virus on the alfalfa plant which in turn increases the opportunity of larvae to contact and ingest the virus.

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Plot	Polyhedra Concentration (Per ml.)	Date (1948)	Active Larvae Per 10 Sweeps <sup>1</sup>		Per cent of Larvae Showing Polyhedra	
			Treated	Untreated	Treated	Untreated
1	97,000,000	86	55	58		
		8–10	100	96	· 100.0	0.0
		8-12	80	84	100.0	0.0
		8-14	10	44	98.0	10.0
		8-17	18	120	100.0	15.0
		8-19	4	145	100.0	75.0
		8-21	4	154	98.0	82.0
2	66,000,000	8–23	6	5		
		8–25	35	38		• • • •
		8–27	24	26		
		8–30	0(4 dead)	16	100.0	0.0
3	57,000,000	9–3	13	19		• • • •
		9–7	32	45		
		9–8	35	41	100.0	15.0
		9_9	8	56	100.0	20.0
4	50,000,000	9–7	5	4		
		9_9	40	41		
		9–13	60	72	98.0	2.0
		9-15	36	174	97.0	13.0
		9–17	47	183	98.0	10.0
		9-21	98	353	95.0	28.0

<sup>1</sup> In order to avoid disturbing the larval population of each plot, the count for each day was based on one sample of 10 sweeps, except that in the case of plot 4, which was considerably larger, the counts were based on an average of 3 samples each.