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Tolerance of Spruce Budworm to Malathion. . . *Montana, New Mexico populations show no differences*

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ABSTRACT: In pest control operations by the U.S. Forest Service using malathion sprays, nearly twice as many spruce budworms were killed in Montana as in New Mexico. To find out if budworms in New Mexico had greater tolerance to the insecticide, malathion was applied by topical and aerosol treatments in the laboratory to 6th - instar populations from the two States. No differences in susceptibility of the insects to malathion were found.

RETRIEVAL TERMS: *Choristoneura occidentalis*; spruce budworm; malathion; insecticides; insecticidal aerosols; toxicity; insecticide-resistant insects; chemical control (insects); insect control; aerosols; Montana; New Mexico.

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Are some spruce budworm (*Choristoneura occidentalis* Freeman) populations more susceptible to the insecticide malathion than others? If so, why? In 1966, U.S. Forest Service pest control specialists sprayed malathion (13 fluid oz./acre) on budworm populations on the Carson National Forest, New Mexico. They achieved 50 percent kill.[1] But the same year, in control work on Montana's Gallatin National Forest and on the Ruby and Greenhorn Ranges, they achieved insect mortalities of 87 and 97 percent with malathion sprays.[2,3]

Three possible explanations for the lower mortality in New Mexico were advanced:[4] (a) the New Mexico budworm may be inherently more tolerant to malathion than the Montana budworm; (b) spray deposit was poor owing to the use of nozzles with larger orifices than customarily used with low-volume sprays; or (c) the exceptionally low temperatures at the time of spraying (32 to 58°F.) may have reduced the effect of the spray.

Some credence was given to the second explanation because droplet density on spray assessment cards was low. But no evidence in support of the third explanation was found by subsequent spray-chamber tests made at two locations on the Carson National Forest, where temperatures ranged from 42°F. or lower to 70°F. and above.[4] At both locations malathion killed an equal number of spruce budworms.

To find out if some budworm populations are more susceptible than others, we applied malathion by both topical and aerosol treatments to 6th-instar spruce budworms in the laboratory. A simple unique method was developed for creating aerosols with an upper diameter limit of about 50µ. Sample populations of spruce budworm came from Montana and New Mexico. We found that populations from the two states showed no differences in susceptibility. Therefore we ruled out the explanation that budworms from New Mexico are inherently more tolerant to malathion than those from Montana.

Spruce Budworm Populations

Fifth and 6th-instar larvae were air expressed[5] to Berkeley, California, from New Mexico, June 15-29, and from Montana July 7-19.[6] The larvae were shipped with fresh host foliage so they could feed in transit and, with few exceptions, were used directly on arrival at Berkeley for testing.

Sixth-instar larvae were treated in groups of 10 and held at 74±4°F. and 45±8 percent R.H. for post-treatment mortality observations in 100 X 20 mm., sterile, plastic, petri dishes. Each dish was lined with a 9-cm. Whatman #1 filter paper. All test insects were fed an artificial diet after treatment.[7]

Both populations contained diseased and parasitized specimens. The number of such specimens was greater among the New Mexico larvae than the other larvae (tables 1,2). The New Mexico larvae harbored a nuclear polyhedrosis and a granulosis virus and a fungus infection, *Beauveria bassiana* (Balsamo) Vuillemin; the Montana larvae were infected with a nuclear polyhedrosis virus only.[8]

All insects used in the tests were weighed and divided among the different concentrations so as to provide each with a sample of insects with about the same weight range and average weight. In the aerosol tests, insects from Montana, averaged 71 mg., and those from New Mexico averaged 72 mg. In tests by topical application, the Montana populations averaged 87 mg., and those from New Mexico 69 mg.

Insecticide Treatment

Malathion was tested in two forms: topical application and aerosol. Topical application was made in acetone with an ISCO Model M microapplicator using a 1/4-cc. tuberculin syringe and 27-gauge hypodermic needle. We applied the insecticide to the dorsum of the thorax of anesthetized (CO_2) larvae at a uniform 1 µl/100 mg. body weight.

The insecticide was tested as an aerosol using a simple but unique method of controlling the upper size limit. Spray was introduced through an orifice 4 inches from the top of a cylindrical glass chamber 15 inches tall and 5-1/4 inches i.d. and allowed to settle on the insects placed at the bottom (fig. 1). The chamber has a removable top for cleaning and an opening at the bottom for introducing insects. This opening is closed during spraying, with a metal plate shaped to the same radius as the chamber.

A #40 DeVilbiss nose and throat nebulizer, modified in Pyrex glass, was used for atomization. An external siphon tube was added to avoid recycling of aerosol as is normal for standard nebulizers. The aerosol was forced through the nebulizer at 5-lb. pressure with a laboratory air pressure pump. Next it passed through a 10-inch long, copper, settling tube to remove large drops, and then entered the chamber. The length of this copper tube determines the upper limit of droplet size. Ten inches allow passage of drops less than 50µ but those more than 50µ settle in the tube before reaching the chamber. The aerosol droplet spectrum was determined with magnesium oxide coated slides as described by May.[9] The slides were placed at the bottom of the aerosol chamber and the droplets that impinged had a maximum drop size of 46µ and an MMD of 19µ.

We treated the insects as follows: 10 unanesthetized insects were placed in a paper food container cover, 9 cm. in diameter, and inserted through the opening in the base of the aerosol

Table 1. Statistical summary of tests with malathion applied topically to the 6th-instar of spruce budworm (*Choristoneura occidentalis* Freeman) from Montana and New Mexico

Statistic	Source of spruce budworm	
	Montana	New Mexico
Median dose at LD_{10}	9.78 ug./gm. bd. wt.[1]	10.0 ug./gm. bd. wt.
Lower and upper 95 percent confidence limits	6.21 to 12.9 ug./gm. bd. wt.	6.03 to 13.5 ug./gm. bd. wt.
Median dose at LD_{50}	29.3 ug./gm. bd. wt.	31.1 ug./gm. bd. wt.
Lower and upper 95 percent confidence limits	24.0 to 34.2 ug./gm. bd. wt.	25.1 to 35.8 ug./gm. bd. wt.
Median dose at LD_{90}	87.9 ug./gm. bd. wt.	97.0 ug./gm. bd. wt.
Lower and upper 95 percent confidence limits	69.2 to 122 ug./gm. bd. wt.	79.1 to 125 ug./gm. bd. wt.
Slope ± SE	2.69 ± 0.23	2.60 ± 0.23
Control mortality	12.5 percent	35.0 percent
Number insects tested[2]	360	420
1-*alpha*[3]	0.31	0.12
Number of concentrations tested	6	7

[1]Dosage expressions refer to active ingredient.

[2]Does not include 40 check insects from Montana and 40 from New Mexico.

[3]*Alpha* = significance level of the chi-square fit test of probit analysis assumptions. *Alpha* near zero indicates assumptions are violated.

Table 2. Summary of tests with malathion applied as an aerosol to 6th-instar spruce budworm larvae (*Choristoneura occidentalis* Freeman) from Montana and New Mexico

Volume of tech malathion[1] (ml.)	Deposit	Total insects	Insects dead after 7 days	Mortality	
				Observed	Corrected[2]
	Fl. oz./acre	Number	Number	— Percent —	
LARVAE FROM NEW MEXICO					
0.0	0	79	31	39	--
.25	6.60	59	49	83	72
.50	14.5	60	55	92	87
.75	23.7	60	56	93	88
1.0	17.4	60	59	98	97
LARVAE FROM MONTANA					
0.0	0	40	4	10	--
.125	2.66	60	12	20	11
.25	5.99	60	25	42	36
.50	13.7	60	46	77	73
1.0	15.8	60	50	83	81

[1]Diluted where necessary in acetone to bring the total volume in the insecticide reservoir to 1 ml.

[2]Abbott's formula.

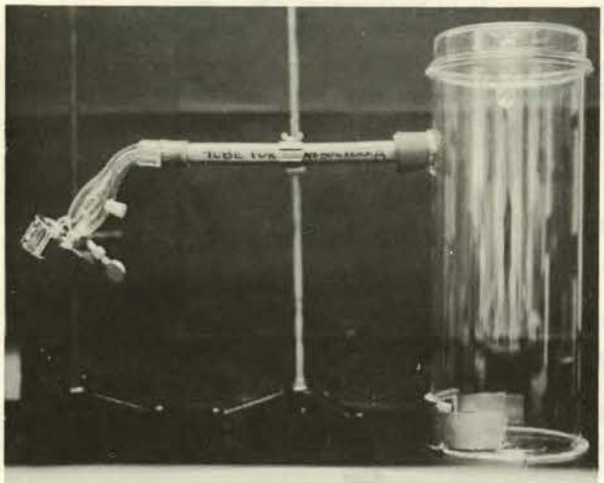

Figure 1.--*Glass aerosol chamber with spray reservoir, nebulizer, and settling tube were used to test the effects of malathion.*

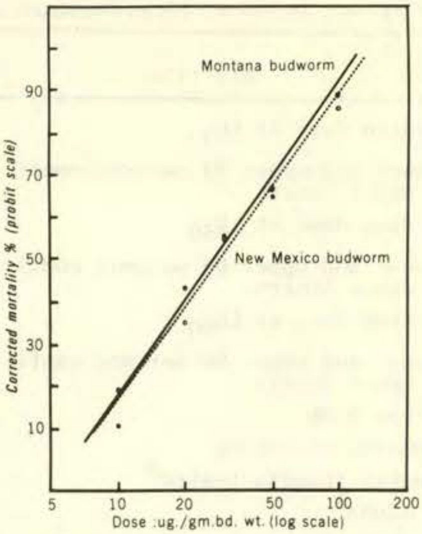

Figure 2.--*Dosage-mortality curves from topical application of malathion to 6th-instar spruce budworm larvae collected from Montana and New Mexico.*

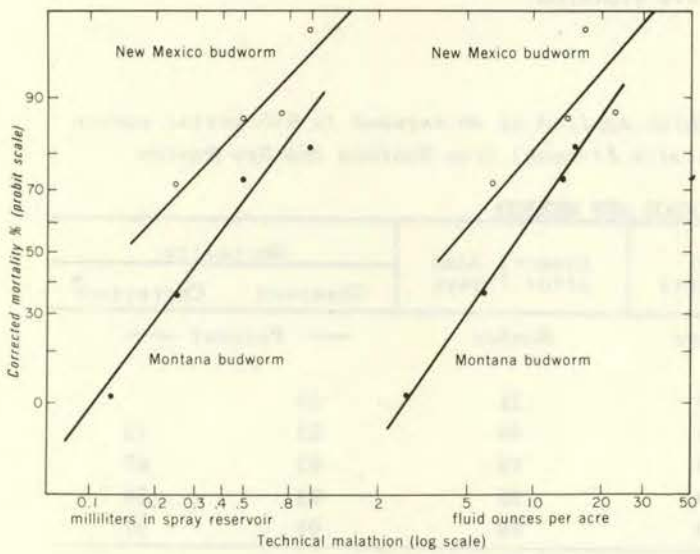

Figure 3.--*Dosage-mortality curves from malathion aerosol (MMD 19μ) applied to 6th-instar spruce budworm larvae from Montana and New Mexico.*

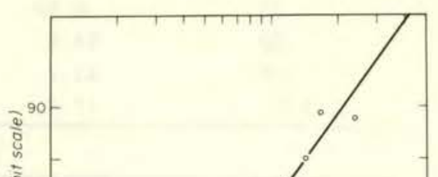

Figure 4.--*Toxicity of aerosol of malathion to spruce budworm from pooled data.*

chamber. We measured the insecticide solution into a small beaker (insecticide reservoir) and exhausted it into the aerosol chamber. The insects were exposed to this visibly turbulent aerosol for 1 minute, then removed to clean petri dishes for 7 days.

A constant volume of 1 ml. of spray solution was placed in the insecticide reservoir for each application. Dilutions in acetone gave us a descending dosage series which could be plotted against mortality. The acetone evaporated when atomized and did not contribute to the deposit. "Deposit" here refers to the amount of malathion that impinged at the base of the aerosol chamber during the 1 minute exposure period. The malathion deposit was estimated, by weight, using 12-mm. diameter aluminum weighing pans and a Cahn electrobalance. The vapor pressure of malathion was low enough not to have affected these estimates.[10] We could detect no weight loss from sprayed pans over a 10-minute period. The aerosol chamber was used in a fume hood and was exhausted of aerosol after each use. The chamber was rapidly purged of aerosol by removing the top of the chamber and the metal access plate at the bottom while the fume hood was running.

Results

In tests by topical application, almost identical numbers of insects were killed in both populations (table 1, fig. 2).[11] The LD_{50} was 29.3 ug./gram body weight for larvae from Montana as against 31.1 ug. for larvae from New Mexico. We found no differences in tolerance to malathion at the 5 percent significance level.

Aerosol tests also showed that the New Mexico budworm was not, in fact, inherently more tolerant to malathion (table 2). The data are presented graphically using two different dosage expressions: volume of technical malathion placed in the spray reservoir and fluid ounces of technical malathion deposited per acre (fig. 3).

Our laboratory data suggest that consistently high levels of kill (95 percent or above) in field practice may require a high dosage of malathion--possibly more than the 1 pound/acre now used. If the data for both budworm populations are pooled, 13 fluid ounces/acre (about 0.94 pound) produced about 80 percent corrected mortality in the aerosol chamber (fig. 4). This dosage is now used for pest control and 80 percent mortality is roughly equivalent to what is obtained on the average in field trials on the spruce budworm. Field trials in 1966 averaged 78 percent. Because of the low slope of the dosage-mortality regression, 90 percent kill required about 20 fl. oz. (about 1.44 lb.). Increments of dosage needed to raise mortality substantially above 90 percent become very large: 95 percent kill required about 30 fl. oz. (about 2.16 lbs.), and 98 percent about 40 fl. oz. (about 2.88 lbs.).

FOOTNOTES

[1]Pierce, Donald A. *Technical phase spruce budworm control with low volume application of malathion Carson National Forest, Region 3, 1966.* 1967. (Unpubl. rep. on file at U.S. Forest Serv., Albuquerque, N. Mex.)

[2]Terrell, Tom T., and Driver, William R. *Mill Creek spruce budworm control project, Gallatin National Forest 1966.* 1967. (Unpubl. rep. on file at U.S. Forest Serv., Missoula, Mont.)

[3]Anonymous. *Spruce budworm control in the Ruby-Greenhorn Ranges, Montana 1966.* 1967. (Unpubl. rep. on file at U.S. Forest Serv., Missoula, Mont.)

[4]Buffam, Paul E. Memorandum in files of U.S. Forest Service, Div. of Timber Management, Region 3, Albuquerque, N.Mex., Dec. 18, 1967.

[5]Under authority of the California Bureau of Plant Quarantine and the Plant Quarantine Division, U.S. Agricultural Research Service.

[6]We thank Jed Dewey and Paul Buffam, who provided the test insects.

[7]Modified after a diet for spruce budworm reported by Arlene McMorran. *A synthetic diet for spruce budworm, Choristoneura fumiferana (Clem.) (Lepidoptera:Tortricidae).* Can. Entomol. 97(1):58-62. 1965.

[8]Determined by Gerard M. Thomas, Division of Entomology, University of California, Berkeley, Calif.

[9]May, K. R. *The measurement of airborne droplets by the magnesium oxide method.* J. Sci. Inst. 27:128-130. 1950.

[10] 4X10-5 mmHg at 30°C.; Negherbon, William O. *Handbook of toxicology*. Vol. III: Insecticides. W. B. Saunders Co. 854 pp. 1959.

[11] We thank Gerald Walton, U.S. Forest Service, Berkeley, Calif., for the dosage mortality regressions which were computed by a modified version of the BMD-03S probit-analysis program (Dixon, W.J. (ed.) BMD 03S biological assay: probit analysis, p. 357-368. *In*, BMD biomedical computer program. Los Angeles: Univ. California School Med. 1964).

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