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DEC 31 1975

TITLE: Project 75-C Insect Pathology *Bacillus thuringiensis* spray research

PERSONNEL: Pinnock, Milstead, Kirby, Nelson, Odom

I. OBJECTIVES & GOALS:

The objective of our 1975 research program (as approved and funded by the Almond Control Board) was to gather basic field data on the persistence of *Bacillus thuringiensis* viable spores. In contrast to Dr. Kellen's approach, we made no concerted effort to control the Transitella larval infestation in the 1975 crop of almonds. Commercial application was purposely delayed 3-4 weeks after the initiation of hull split to allow us to complete the persistence trials!

We felt that for a proper evaluation of the effectiveness of *B.t.* for the control of the Transitella moth, it is essential that field data be secured on: 1) the dosage-mortality relationships existing between commercial formulations of *B.t.* and early instar Transitella larvae; 2) the magnitude and variability of initial deposits of viable spores on almond hull tissue following high and low volume application of commercial formulations of *B.t.*; and 3) the persistence of the pathogenic components (spores, toxic crystals) of commercial formulations of *B.t.* on both internal and external almond hull tissue. This information, along with data on ovipositional rhythms, can provide a basis for the selection of those commercial formulation(s), concentrations and spray schedules that would be most effective in the control of infestations of the Transitella moth in almond orchards.

II. ABSTRACT:

Utilizing a modification of a standard method developed by Dr. Pinnock for the assay of viable spores on foliage, we have determined the persistence half-life of spores deposited both on the external surface of the hull and

upon the exposed internal tissue at time of hull split. These values (1 1/2 and 5 days respectively) represent the length of time required for one-half the original number of spores to become inactivated.

We have also determined that in spite of the enormous variability in initial coverage an average dosage of 684 international toxicity units/ $50mm^2$ of hull tissue produced a mortality of approximately 60%. While we remain optimistic with regard to the potential of this ecologically gentle microbial insecticide for Transitella moth control, this study indicates that a single high volume applicaion delayed 3-4 weeks after initiation of hull-split will not result in a substantial reduction of infestation levels at harvest time.

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III. Experimental Procedure:

Commercial formulations of *Bacillus thuringiensis* contain viable spores and toxic protein crystals both of which interact to produce pathogenesis in lepidopterous larvae. The stability of the spores is influenced by many environmental factors, the most important of which are solar radiation and substrate (Pinnock, et al., 1975; 1976 in press).

The dose to which the target insect is exposed is dependent both upon variability of initial coverage and subsequent decay rates of toxic components prior to ingestion. Coverage and persistence are simply and reliably ascertained by determination of the density of the viable spores in samples taken at, and subsequent to, the time of treatment.

Viable spore population density was assessed by methods previously developed for determining the field persistence of viable spores on leaf surfaces of various species of trees and shrubs (Pinnock, et al., 1971; 1974; Brand et al., 1975). Discs of almond hull tissue (50 mm^2) were excised using a sterile No. 4 brass cork borer. Discarded surfaces were removed by slicing with a sterile scalpel blade. Tissue segments were transferred to culture tubes containing 10 ml. of sterile distilled water, closed with sterile amber rubber stoppers and agitated at 766-16 mm. strokes/min. on a modified Burell wrist action shaker. The suspensions are then mixed by centrifugal action with a Vortex Genie mixer and the 10 and 100 fold dilution plated, incubated, and counted on Difco^R brain heart infusion agar (Miles & Misra, 1938).

A. Persistence and Coverage.

Field trials in the Swanson orchard, Capay, California, were initiated in June and continued through September. Four external and three internal persistence trials based upon 716 individual nut samples from 40 trees were completed. The results of these trials are reported in Tables 1 & 2. In addition estimates were obtained on the initial internal (within hull) deposition of viable spores on 60 nuts from 50 trees treated with 3 commercial B.t. formulations. These are reported in Table 3.

B. Oviposition Monitoring.

Oviposition was monitored utilizing media-filled 13 dram plastic cages hung in the orchard at elevations of 4-8 ft. above ground level. The cages were modified by providing two symmetrically positioned holes (9.8 cm^2 total area) covered with 44 x 36 count cheesecloth. The media volume was approximately 15 cc. Between 4/8 and 7/3/75, 21 oviposition trials (929 cages) were made comparing unused diet, used diet (previously used in Transitella larval rearing), stick tight (mummy) nuts and unfilled cages (blanks). Three additional trials were completed between 7/3 and 8/22/75 comparing ground Nonpareil almonds, used diet and a 1:1 combination of both. The results of these trials are reported in Table 5.

IV. Results & Discussion

A. External Persistence.

The external persistence half-life, π, (the average viable spore count at time $t + \pi = 1/2$ the average viable spore count at time t) varied between 0.77 and 4.62 days and was dependent upon the position of the sampled tissue relative to the incident solar radiation. The lowest value

was obtained from almonds whose treated surfaces were oriented toward the sun, the highest from tissue oriented away from the sun. The degree of exposure was further modified by protective shading from foliage, branches and other nuts.

B. Internal persistence.

The internal persistence varied between 2.2 and 9.3 days. In addition to nut orientation and protective shading, it is possible that physiological characteristics of the internal hull tissue (a function of hull-split age) exert an influence on spore stability.

A bioassay was incorporated into the design for the internal persistence trial of 8/26-9/2/75. A group of sixty 3rd and 4th stage larvae (selected from a laboratory stock colony previously reared on a diet of bran, honey, and glycerin) served as test animals. These were challenged with 50 mm^2 discs excised from almond hulls at 0 and 7 days post-treatment (0.75% Thuricide HPC). The initial dosage, averaging 5.5×10^5 spores/disc with a range of 1.1×10^4-3.6×10^6 resulted in a control-corrected mortality of 25%. The 7-day post-treatment sample averaging 4.8×10^5 spores/disc with a range of 5.6×10^3-1.3×10^6 resulted in an uncorrected mortality of 10%. During this experiment comparisons between control and Bt-exposed larvae suggested no Bt-induced feeding inhibition.

These data indicate that B.t. deposited on the hull interior can still cause mortality in 3rd-4th stage Transitella larvae at least one week after treatment. First instar larvae are undoubtedly more susceptible (from dosage/body weight ratio considerations) but exact correlations are unknown.

C. Commercial coverage.

Viable spore counts following commercial treatment (Table 3) reveal

excessively high variability of coverage that was influenced by heterogeneity in nut orientation and degree of hull split as well as effectiveness of action of the foliage as a barrier to penetration. Dipel gave unexpectedly low spore deposition. The mean International Units of toxicity/disc (extrapolated from label data) for the three commercial B.t. formulations was 684. This level of dosage would be expected to produce a mortality in excess of that indicated in the laboratory bioassay. Samples of almonds collected by Dr. Leo Caltagirone at 17 and 36 days post-treatment revealed larval mortalities of 57.1% and 62.1% respectively. These larvae, as well as larvae from samples submitted to this laboratory in mid-November gave positive diagnoses indicative of B.t.-induced mortality.

The results of the "commercial" spraying are reported in Table 4. The Transitella larval infestation levels of Thuricide-treated almonds was lower than that of the controls in both sets of samples, significantly less (P=0.01) at 35 days post-treatment. The commercial application was delayed 3-4 weeks after onset of hull split in order that the persistence trials migth be completed. This delay (at a peak time of ovipositional activity) as well as the low dosages and inadequate coverage may account for the high levels of infestation in B.t.-treated samples.

D. Oviposition monitoring.

The results of the oviposition monitoring trials are reported in Table 5. The cages appeared to be ineffective during early season monitoring prior to hull split. This may be a function of cage design, media formulation, or competition with natural oviposition sites. The combination of nuts and

used diet appeared to be the most promising media for eliciting ovipositional response.

Research conducted during 1975 has shown that the commercial formulations of B.t. can induce appreciable mortality in Transitella larvae following a single application at 3-4 weeks following initiation of hull split. The external and internal persistences of Thuricide HPC are of the order of 1.5 and 5.0 days respectively. This would seem to indicate that an early application at the onset of hull split followed by applications at 7-10 day intervals (at concentrations and rates designed to produce a mean of approximately 1000 I.U./50 mm^2 disc) would be efficacious in reducing Transitella infestations to economic levels.

Bibliography

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TABLE 1. *Bacillus thuringiensis* External Persistence on Almond Hulls--Swanson Orchard, Capay

Variety	Date	Formulation	Concentration (%)	Total Nuts Sampled	a Intercept	b slope	$T_{\frac{1}{2}}$ half-life (days)
Nonpareil	6/26-7/3/73	Thuricide HPC	0.75%	18	5.86	-0.138	2.17
Nonpareil	7/17-24/75	Thuricide HPC	0.50	68	5.96	-0.363	0.82
Texas	7/17-24/75	Thuricide HPC	0.50	167	6.03	-0.200	1.50
Texas	7/25-31/75	Thuricide HPC	0.50	40	5.85 (1)	-0.392 (1)	0.77 (1)
				40	5.98 (2)	-0.065 (2)	4.62 (2)

(1) Upper surface; i.e., surface oriented towards sun.

(2) Lower surface; i.e., surface oriented away from sun.

TABLE 2. *Bacillus thuringiensis* Internal Persistence within Almond Hulls--Swanson Orchard, Capay

Variety	Date	Formulation	Concentration (%)	Total Nuts Sampled	a Intercept	b Slope	π Half-life (days)
Nonpareil	7/20-24/75	Thuricide HPC	0.5%	18	5.83	-0.086	3.51
Nonpareil	7/28-8/6/75	Thuricide HPC	0.5%	191	6.13	-0.136	2.22
Peerless	8/26-9/2/75	Thuricide HPC	0.75%	176	5.62	-0.032	9.32

TABLE 3. Internal (within hull) deposition of viable spores following treatment (1) on 8/4/75 with commercial formulations of Bacillus thuringiensis.

Formulation	Spores/gal.	I.U./gallon	viable spore count (2) spores/disc		I.U./disc	
			$\bar{x}$	range	$\bar{x}$	range
Biotrol XK	3.8×10^{10}	2.4×10^{8}	2.58×10^{5}	$1.2 \times 10^{4} - 8.8 \times 10^{5}$	1610	72.5 - 5000
Dipel WP	3.8×10^{11}	2.4×10^{8}	1.68×10^{5}	$1.2 \times 10^{4} - 6.9 \times 10^{5}$	107	7.4 - 439
Thuricide HPC	3.6×10^{11}	2.4×10^{8}	5.04×10^{5}	$5.6 \times 10^{3} - 1.6 \times 10^{6}$	336	3.7 - 1070

(1) John Bean FMC Sprayer. Rate: 450 gallons/acre at 1 MPH

(2) Sample size: 20 nuts/treatment

TABLE 4. Transitella larval infestation in Nonpareil almonds collected 7 and 35 days after treatment with commercial formulations of Bacillus thuringiensis. Swanson Orchard, Capay

Treatment	Days post-treatment	I.U./acre	Number sampler(1)	$\bar{x}$	s
Control	7	--	5	16.3	11.2
	35		15	24.6	4.1
Thuricide	7	3.6×10^{11}	5	5.8	2.7
	35		15	20.8	2.7
Biotrol	7	3.6×10^{11}	5	23.3	7.8
	35		15	24.0	2.2
Dipel	7	3.6×10^{11}	3	31.2	5.3
	35		13	23.1	9.8

(1) 1.5 lbs. in shell nuts/sample

TABLE 5 Transitella moth Oviposition 1974--Swanson Orchard, Capay

Date	Total trials	Media in cages	Total Cages Exposed	No. of cages With Tm eggs	% cages With Eggs
4/8-7/3	21	Unused diet	250	19	7.6
	21	Used diet	250	26	10.4
	21	Stick tights	250	3	1.2
	18	Blanks	179	1	0.6
7/3-8/22	4	Used diet	118	22	18.6
	4	Ground almonds	117	29	24.8
	3	Ground almonds + used diet	81	27	33.3