

bi-monthly research notes

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INSECT PATHOLOGY

Two Entomopoxvirus Strains Isolated from the Spruce Budworm, *Choristoneura fumiferana* (Clem.).—Entomopoxviruses (EPV's) have morphological similarities to vertebrate poxviruses but differ significantly in their genomic structure and protein composition (Arif, *Virology* 69:626-634, 1976). Moreover, EPV's are occluded in a proteinaceous mass and are transmitted in this occluded form.

EPV's were first described by Vago (*J. Insect Pathol.* 5:275-276, 1963) and subsequently several were isolated from Diptera, Orthoptera, Lepidoptera and Coleoptera (Bergoin and Dales, pages 169-205 in Maramorosch and Kurstak, eds., *Comparative virology*, Plenum Press, 1971). An EPV was isolated from the 2-yr-cycle spruce budworm *Choristoneura biennis* Free. by Bird (*J. Invertebr. Pathol.* 18:150-161, 1971); and, more recently, J.M. Burke isolated an entomopoxvirus from *C. fumiferana* (Clem.) that possessed some very large virus inclusion bodies (VIB's). On close examination there appeared to be two distinct populations of virus inclusions in infected larvae. The two types of inclusion bodies did not coexist in any one infected cell, and this indicated that there may be two virus strains in the original isolate from *C. fumiferana*. One type of VIB (strain I) is oval and larger than the other (strain II), which is more angular and produces a larger number of inclusions per cell (Fig. 1).

These virus isolates were propagated in second-instar larvae reared on artificial diet. Each diet cup received 10^5 VIB's and the larvae were incubated for 25-30 days. Each larva was diagnosed individually for EPV infection, and the progeny virus was passaged in additional larvae; this was done by extracting the VIB's from individual insects. The concentration was adjusted to 2×10^5 VIB's/mL and 0.5 mL was added to each diet cup. With continuous virus passage in larvae the relative amount of strain II over strain I virus increased. After 7-10 passages no strain I VIB's were detected in infected larvae; this indicated that strain II virus is more virulent. When both strains were propagated in the same insect, strain II interfered with the multiplication of strain I virus and eventually eliminated it. This is further supported by the fact that a pure strain I will always produce strain I progeny regardless of the number of passages in larvae. Likewise strain II gives rise to strain II progeny only.

The inclusion bodies of both strains were semipurified by differential centrifugation in a Sorval centrifuge, and their size was estimated from phase-contrast photomicrographs. The size of strain I VIB's, from measurements of 121 inclusions, is $7.87 \pm 0.29 \mu\text{m} \times 11.25 \pm 1.6 \mu\text{m}$; a few inclusion bodies as large as $12.2 \times 17.7 \mu\text{m}$ and as small as $5.4 \times 7.1 \mu\text{m}$ were observed. The size of strain II inclusions by comparison is much smaller. From measurement of 111 inclusions the size was found to be $3.4 \pm 0.55 \mu\text{m} \times 4.31 \pm 0.7 \mu\text{m}$; the largest and the smallest strain II inclusions were $4.8 \times 5.3 \mu\text{m}$ and $2.4 \times 3.4 \mu\text{m}$. Although there is considerable variability in the size of strain I inclusions and, to a lesser extent, in those of strain II, the shape of each is characteristic.

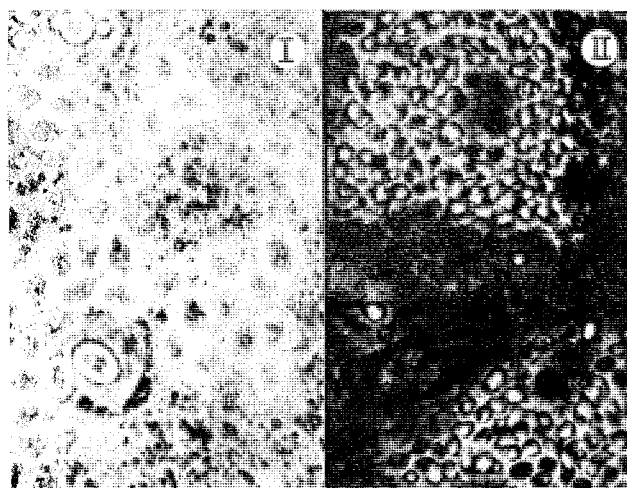


Figure 1. *C. fumiferana* cells infected with strain I and strain II entomopoxvirus.

It is conceivable that these two strains of virus exist separately in nature in different spruce budworm populations and that, when these populations merge, a double infection occurs. The more virulent virus will then interfere with the multiplication of the less virulent strain and will either reduce its proportion drastically or eventually eliminate it.—B.M. Arif and Keith W. Brown, Forest Pest Management Institute, Sault Ste. Marie, Ont.

ENTOMOLOGY

Pine Oil Prevents Mountain Pine Beetle Attack on Living Lodgepole Pine Trees.—Pine oil sprayed on bark surfaces of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) log sections delayed and reduced attacks by ambrosia beetles and appeared also to reduce bark beetle attacks (Nijholt, *Bi-mon. Res. Notes* 35:22-23, 1979; Nijholt, *Can. Entomol. in press*).

As an extension of the foregoing, an experiment to determine the effect of pine oil on attacks by mountain pine beetle (*Dendroctonus ponderosae* Hopk.) on living lodgepole pine trees (*Pinus contorta* Dougl.) was carried out in a 140-yr-old primarily lodgepole pine stand east of McLeese Lake, B.C., in the summer of 1979.

Twenty uninfested pine trees were selected in an area adjacent to a natural infestation of mountain pine beetle. The lower 2.4 m of the stems of 10 trees were sprayed to the drip point with undiluted Norpine 65 (pine oil, supplied by Northwest Petrochemical Corporation, Anacortes, Wash., 98221, U.S.A.) with a garden-type pressure sprayer (Hudson Manuf. Co. Model #6622) on 5 July 1979. The other 10 trees were left as untreated controls.

Each of the 20 trees was then baited at breast height with two caps containing 0.5 mL of a mixture of *trans*-verbenol and alpha-pinene (9:1) (Pitman, *J. Econ. Entomol.* 64:426-430, 1971) and with 5 mL of 95% ethanol in a loosely capped polyethylene Boston bottle to take advantage of possible synergistic effects (Pitman et al., *Z. Angew. Entomol.* 78:203-208, 1975).

The trees were checked daily from 19 to 30 July, inclusive, and on 14 and 21 August. The first attacks were observed on 23 July on untreated trees. By the end of the experiment, on 31 August, when beetle flight had ended, 8 of the 10 untreated trees were heavily attacked. The remaining two received zero and two attacks, respectively. Nine of the pine oil treated trees remained free from attack. The 10th had 15 attacks above the treated part of the stem and six within the treated area; all galleries in this tree were pitched out.

No evidence of phytotoxicity owing to the treatment was observed on the trees by 9 October 1979, although damage occurred to underbrush near the stem of the treated trees. The pine oil, as applied, was effective in reducing attacks by the mountain pine beetles. The trees

will be kept under observation for evidence of resistance to future beetle attack and of phytotoxicity.—W.W.Nijholt and L.H. McMullen, Pacific Forest Research Centre, Victoria, B.C.

Field Test of Swedish "Drainpipe" Pheromone Trap with Mountain Pine Beetle.—The mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is currently causing serious damage to lodgepole pine (*Pinus contorta* Dougl.) forests in British Columbia. Present control efforts are restricted largely to salvage logging of infested stands. Population and damage reduction is achieved only if green infested trees are cut and the brood is destroyed (Safranyik et al., Environ. Can. For. Tech. Rep. 1, 1974). The use of attractive pheromones for bark beetle mass trapping has been investigated in several species, including the mountain beetle in white pine (Pitman, J. Econ. Entomol. 64(2):426-430, 1971). In 1979, a massive control program against the European spruce beetle *Ips typographus* L. was undertaken in Norway and Sweden, in which, respectively, 600,000 and 350,000 pheromone-baited traps were deployed in areas where trees were being killed. The aim of the program was to reduce the beetle population to a level below the economic threshold (O'Sullivan, Chem. Eng. News, 57(31):10-14, 1979). The objective of the study reported here was to determine if traps of the type produced in Sweden for *I. typographus* could be used to trap mountain pine beetle in lodgepole pine stands.

The trap (Fig. 1) consisted of a piece of corrugated black-plastic drainpipe 1.45 m long and 11.5 cm in diameter. Six evenly spaced longitudinal rows of holes 5 mm in diameter (714 in all) were drilled through the pipe wall between the corrugations. A white-plastic funnel and a 2 L plastic widemouthed jar were clamped to the bottom of the pipe. A black-plastic cap at the top of the pipe served to keep out rain. In principle, the pipe simulated a tree-stem silhouette; the holes in the pipe served as exits for the bait pheromones placed inside the trap and as entrances for the attracted beetles that then fell into the jar.

Seven traps were deployed at 7 to 10 m intervals, six in a circle and one at the center, in an infested stand at Riske Creek, B.C., from 17 to 26 July, 1979, inclusive. They were tied with wire to stakes driven into the ground, so that the bottom of the bottles was approximately 30 cm above the ground. Two polyethylene caps, each containing approximately 0.25 mL of "pondelure" (9 parts *trans*-verbenol, 1 part α -pinene) as bait, were suspended at two levels inside each trap. Pondelure is acknowledged to be a poor attractant for mountain pine beetle on traps in lodgepole pine stands (Pitman et al., pages 165-173 in Kibbee et al. [eds.], Theory and practice of mountain pine beetle management in lodgepole pine forests, Univ. Idaho, Moscow, 1978). Therefore, other materials, as follows, were added to the bait in five of the traps. Each of two traps had a small fresh lodgepole pine bolt (8 x 50 cm), manually infested with 15 female mountain pine beetles and screened (Fig. 2), as a natural source of pheromones. In each of the three remaining traps were placed two loosely capped polyethylene Boston bottles (5 mL). These were filled as follows: the first pair with 95% ethanol (Pitman et al., Z. Angew. Entomol. 78(2):203-208, 1975) (Fig. 3); the second pair with acetone (Billings et al., Environ. Entomol. 5(1):171-179, 1976); and the last pair with ethanol in one case and

TABLE 1

Catches of mountain pine beetle on pheromone-baited Swedish "drainpipe" traps

		Number of <i>D. ponderosae</i> caught					
Trap no.	Bait	Pipe trap		Sticky screen		Total	Sex ratio
		♂	♀	♂	♀		
1	Pondelure	4	11	1	1	17	1:3.0
2	Pondelure	2	24	2	7	35	1:7.8
3	Pondelure + ethanol	3	3	7	7	20	1:1.0
4	Pondelure + acetone	3	7	0	4	14	1:3.7
5	Pondelure + ethanol + acetone	5	9	0	6	20	1:3.0
6	Pondelure + ♀-infested bolt	15	15	3	4	37	1:1.0
7	Pondelure + ♀-infested bolt	2	11	2	1	16	1:3.0
	Totals	34	80	15	30	159	1:2.4



Figure 1. Swedish "drainpipe" trap in operation.

Figure 2. Female-infested pine bolt, with pheromone-containing caps below.

Figure 3. Polyethylene cap with 1/4 mL pondelure, and Boston bottle with 95% ethanol.

acetone in the other. To determine if beetles were arriving at the trap but not entering it, a sticky screen cylinder 17 cm in diameter and 22 cm high was attached near the top of each trap (Fig. 1); the ratio of surface areas of the pipe trap (minus the area obscured by the screen) and screen was 3.8 to 1. The traps were checked seven times in the 10-day period.

Although the number of beetles caught was small (Table 1), the test does indicate that the pipe trap is suitable for dead-trapping mountain pine beetles. However, the number of beetles that attacked all surrounding pines, some as small as 8 cm dbh, far exceeded the number