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bi-monthly research notes

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ENTOMOLOGY

Laboratory Methods for Rearing the Forest Tent Caterpillar.—Large numbers of forest tent caterpillar [*Malacosoma disstria* Hbn.] have been reared throughout the year at the Insect Pathology Research Institute since 1966 for insect pathogen and physiological investigators. Larvae are reared entirely on artificial food. The artificial food and its method of preparation are described by Grisdale (Can. Entomol. 105:1553-1557, 1973). Raw linseed oil, 015 ml per 100 ml of diet is an essential ingredient because without it many adults have crippled wings.

Most of the eggs used are field-collected during mid-September and they are placed immediately in storage at a temperature of 0° to 2°C. Every effort is made to collect from areas of new infestation where disease and parasitism are usually at a low level. Eggs are usually removed from cold storage after a minimum of 4 months; hatching commences after 5 days of incubation. Optimum time for egg storage is 6-9 months; eggs so treated commence hatching in 2 or 3 days with maximum hatch rate occurring over a period of 2 or 3 days. Eggs may be stored for 12 months but this prolonged storage increases the incubation time to 5 or 6 days, reduces the hatching rate, and up to 40% of the egg bands do not hatch. Eggs from laboratory reared adults are produced to supplement rearings during the period when field material is unsatisfactory.

To phase larval rearings so that all stages are available for research at any required time, egg bands are removed from cold storage at weekly intervals. The egg band is removed intact from the twig and soaked in a sodium hypochlorite solution (the household bleach, Javex®, which has 6% available sodium hypochlorite) to remove the frothy spumilin covering and surface virus (Grisdale, J. Invert. Path. 10:425, 1968). If fewer larvae per rearing unit are required, the egg band may be cut into sections with surgical scissors. When the first few larvae are observed the egg band is placed into a ¾ oz. plastic creamer cup containing approximately 15 ml of diet, capped and inverted so that the egg band rests on the cap and does not come into contact with the surface of the diet. Cups are exposed to 16 hours illumination, temperature of 23 ± 1°C and relative humidity of 60%.

Newly emerged larvae leave the egg band, climb the wall of the cup and readily establish on the artificial food. Most of the larvae reach the third instar in approximately 12 days. The cups at this stage appear densely packed; however, forest tent caterpillar larvae seem to thrive and develop better under crowded conditions. Larvae are then thinned to 10 per cup and the cups again placed in an inverted position until the last instar is attained (usually six instars when reared on artificial food under laboratory conditions). Last instar larvae are transferred to well ventilated 30x38x15 cm plastic crisper trays lined with paper towelling. A portion of diet sufficient to last 24 hours is placed on a piece of aluminum foil on the bottom of the tray. The foil prevents the diet from loosing moisture to the paper liner. Last instar larvae of the same size required for experimentation are easily selected from the trays. Larvae to be reared through the adult stage for sex pheromone studies or for rearing stock are transferred to cups containing a small amount (6 ml) of diet. Cups are placed in an upright position and spinning and pupation occur in the underside of the lid. Pupation commences approximately 30 days after larval hatch. Pupae are separated from the cocoons and lids by soaking them in a sodium hypochlorite solution as described by Grisdale (Bi-mon. Res. Notes 31:9, 1975) and are sexed as described by Muggle (Ann. Entomol. Soc. Am. 67:521-522 1974). Males are initially held 4°C cooler than females until adult emergence is synchronized.

Adults, which emerge approximately 2 weeks after pupation, are mated in 1-pint cylindrical cardboard containers. A short section of a trembling aspen branch tip with a few lateral twigs is placed in the container as oviposition sites. A petri dish, which allows observations of mating and egg deposition, is used to cover the mating chamber. Better mating success and egg production occur when adults, particularly the females are mated as soon after eclosion as possible. Adults for which there are no mates may be held in the refrigerator for a day or two. Mating containers are held at 20±1°C and relative humidity 90–95%. Two pairs of adults per mating unit gives maximum egg production as evidenced by recent mating and oviposition tests. Nine matings using two pairs of adults per mating unit yielded 18 egg bands or 100% mating success, whereas thirteen matings using three pairs of adults yielded only 21 egg bands or 63.7% mating success. Eggs are usually laid within 2 days of mating. After 5 weeks of pre-storage incubation the egg bands are placed in a cardboard mailing tube, sealed and placed in cold storage. The fertility of the egg bands may be checked by dissecting a few eggs before storage to see if first instar larvae are present.

In one test, all the eggs hatched in a sample of 14 laboratory reared egg bands storage for 7 months. With are method hatching varies from 11.6-94.7% among egg bands with an overall average of 77.9% and an average of 139 larvae are recovered from each egg band.—D. G. Grisdale, Insect Pathology Research Institute, Salt Ste. Marie, Ont.

Forest Tent Caterpillar Moths Found in Newfoundland.—The forest tent caterpillar [*Malacosoma disstria* Hbn.] periodically causes severe defoliation of trembling aspen [*Populus tremuloides* Michx] in Canada and northern United States. The insect occurs throughout the Maritime Provinces but not on the island of Newfoundland. However, in 1951 one adult was collected in a light-trap near Lake St. George, Newfoundland (Can. Dep. Agric., For. Ins. Dis. Surv., 1952), and was identified as a male (Carroll, personal communication). No other specimens have been seen on the Island until eight male moths were collected at lights at Pasadena on 19 July 1975. No additional moths were found in the surrounding areas during the next search 4 days later, and a survey of aspen stands within 3–5 km of the collection point revealed no infestations.

It is suspected that the moths were carried to Newfoundland by air movements, such as up-drafts associated with moving cold fronts. The nearest source from which the moths may have originated is Cape Breton Island, Nova Scotia, about 250 km away. Mass transport of forest tent caterpillar adults by a cold front for a distance of 480 km has been documented in central Alberta (Brown, Can. Entomol. 97: 1073-1075, 1965). In 1968 I checked several thousand moths which had been transported by air currents for about 80 km

in Alberta and all were males. The long distance transport of females by air movements is not known, therefore infestations of forest tent caterpillars are unlikely to occur in Newfoundland from moths brought in by this method.

General sampling by the Forest Insect and Disease Survey did not indicate the presence of the forest tent caterpillar on the Island in 1975, but a special survey is planned for 1976.—A. G. Raske, Newfoundland Forest Research Centre, St. John's, Nfld.

Greenhouse Evaluation of PH 60-40 Activity on the Forest Tent Caterpillar.—The forest tent caterpillar, [*Malacosoma disstria* Hbn.] is a serious defoliator of hardwood trees and sporadic outbreaks occur in various parts of Canada. Preliminary investigations utilizing forest tent caterpillars maintained on artificial diet (Grisdale, Can. Entomol. 102:1111-1117, 1970) indicated that an insect growth regulator (IGR), PH 60-40, was very effective in inducing lethal abnormalities at relatively low concentrations.

PH 60-40 is a substituted phenyl urea that interferes with chitin biosynthesis when ingested, and its effects are manifested at the time of moulting (Post and Vincent, Naturwissenschaften 6.:431-432, 1973). This compound is highly insoluble in water and has virtually no contact effect. It is resistant to shortwave ultraviolet, is not easily leached, and persists on foliage for at least 20 days (Retnakaran and Smith, Can. Entomol. 107:883-886, 1975). No apparent side effects on selected nontarget species have been detected. It was felt that this IGR might be a good candidate for controlling forest tent caterpillar populations in the field. In this report we present results of field simulation studies conducted in the greenhouse with this and two other IGRs.

Potted trembling aspen [*Populus tremuloides* Michx.] about 60 cm high were placed individually in a spray tower equipped with a fine nozzle (Micro II_a–19/32) and were treated with a 1% solution of the test compound at the rate of μ1/tree (dosage calculated to be equivalent to 1 U.S. gallon/acre). Details of the procedure were published earlier (Retnakaran and Smith, Can. Entomol. 107:883-886, 1975). The three IGRs tested were ZR-515-5E (Zoecon Corp., Palo Alto, Calif.), R-20458-4E (Stauffer Chemical Co., Mountain View, Calif.), and PH 60-40 (Philips Duphar Ltd., Amsterdam, Holland). The first two compounds are juvenile hormone analogues and interfere with metamorphosis (Weatherston and Retnakaran, J. Environ. Qual. 4:294-303, 1975). Trees (two per treatment) were sprayed with the candidate compound and 10 fourth instar larvae were placed on each tree. The trees were enclosed in a screened Plexiglas container and placed in the greenhouse.

TABLE 1

Effect of some insect growth regulators on *Malacosoma disstria* larvae on potted trembling aspen trees under greenhouse conditions

Treatment	No. larvae/tree	Replicates	% preemergence mortality
Control	10	2	34
R-20458-4E (Stauffer)	10	2	55
ZR-515-5E (Zoecon)	10	2	90
PH - 60-40 (Philips-Duphar)	10	2	100

Abnormal differentiation was observed in all treatments. R-20458-4E was the least effective; ZR-515-5E extended the larval stage. In the PH 60-40 treatment preemergence mortality was 100% (Table 1). A field evaluation of the efficacy of PH 60-40 on the forest tent caterpillar on a limited scale is contemplated.—A. Retnakaran and L. Smith, Insect Pathology Research Institute, Sault Ste. Marie, Ont.

A Preliminary Field Trial Using *Nosema fumiferanae* against the Spruce Budworm, *Choristoneura fumiferana*.—The spruce budworm hosts a number of pathogens including a microsporidian *Nosema fumiferanae*. This parasite has been shown to adversely affect the vigor and fecundity of the host (Thomson, Can. J. Zool. 36:499-511, 1958), as well as cause mortality under laboratory conditions (Wilson, Can. J. Zool. 52:993-996, 1974). Preliminary results of spray trials indicate that *N. fumiferanae* can be successfully introduced into a field population of spruce budworm and produce higher infection rates than occur naturally.

Microsporidian spores for experimental application were produced by inoculating laboratory-reared spruce budworm larvae with the parasite, and were subsequently harvested by homogenizing the infected larvae in distilled water. The homogenate was passed through double-thickness cheesecloth to remove large pieces of debris. The spores were allowed to settle for 3 days, after which most of the supernatant was removed to reduce the volume of material to be stored. The spore suspension was refrigerated at 4°C for 1-4 months. Before field trials, the spore samples were pooled and mixed in water and the spore concentration was determined by using a hemacytometer. A formulation of 12% microsporidian suspension (1×10^9 spores/ml) in distilled water (v/v) was prepared and sprayed at a rate of 1 500 ml per tree.

The field site was predominantly occupied by white spruce (*Picea glauca*) 3.2-7.4 m in height, near the town of Burpee, on Manitoulin Island, Ontario. Ten trees were selected for spraying, and five check trees were chosen in an area close to the treatment trees to ensure that levels of natural infection would be the same in both.

Spraying was carried out on the evening of June 2, when weather conditions were suitable, with a packsack-type sprayer (Ken-San Ltd. KWH 2677). At the time of spraying, larval development was predominantly at the IV instar. Incidence of the microsporidian parasite in the spruce budworm population was monitored by a prespray and two postspray samples. These samples consisted of 46-cm branch tips from the mid-crown of each sample and check tree. Larvae were removed from the samples, and smears prepared for each larva were examined under phase contrast for the presence of *N. fumiferanae*.

The results of spraying on the incidence of *N. fumiferanae* in spruce budworm larvae are shown in Table 1. The prespray sample indicates that 13.5% of the larvae were naturally infected with *N. fumiferanae*. Budworm larvae from sprayed trees taken 11 and 25 days after spraying had significantly higher levels of infection than budworm from the check area. In general, there was a progressive increase in the levels of *N. fumiferanae* as the time after spraying increased. However, this was true of checks as well. This phenomenon has been observed in budworm populations naturally infected with *N. fumiferanae* (Wilson, Bi-mon. Res. Notes 29:35-36, 1973).

TABLE 1

Incidence of *Nosema fumiferanae* in living spruce budworm larvae collected from sprayed and check spruce trees on 2 June 1975

Trees	Sample date	No. insects examined	Incidence of *N. fumiferanae* (%)
Treatment area	May 26 (Prespray)	613	13.5
Treated	June 13	516	45.9**
check	June 13	52	17.6
Treated	June 27	568	53.0**
check	June 27	174	23.0

** These values are significantly different from the controls at the 1% level; t-test as applied to percentages.

These results indicate that *N. fumiferanae* can be successfully introduced into a population of spruce budworm and thereby increase the levels of the microsporidia in that population. Since the pathogen is transmitted transovarially, most offspring of infected adults are also infected. The effect on adult longevity and fecundity, as well as on larval and

pupal development, could possibly play an important role in controlling the levels of spruce budworm populations. It is hoped that further field trials will establish the efficacy of *N. fumiferanae* as a biological control agent of the spruce budworm.—G. G. Wilson and W. J. Kaupp, Insect Pathology Research Institute, Sault Ste. Marie, Ont.

Effect of Insect Growth Regulators on the Survival of Douglas-fir Beetle Progeny.—Insect growth regulators (IGR) have shown promise at the operational level against mosquitoes and at the experimental level, under field conditions against insect pests of livestock and some forest defoliators (Staal, Annu. Rev. Entomol. 20:417-460, 1975). Scolytid and curculionid coleopterons were, however, considered to be insensitive to such materials (Slama, Annu. Rev. Biochem. 40:1079-1102, 1971). This report describes the effects of a variety of IGR, particularly ZR-515 (Zeocon Corp., Palo Alto, Calif.), on the development and mortality of Douglas-fir beetle broods [*Dendroctonus pseudotsugae* Hopk.].

For this study, the beetles were collected from the field after they had overwintered. The host material was presented to the beetles in the form of a 150-mm-dia bark and phloem slab in a plastic petri dish. The slab was clamped in the petri dish so that the phloem was held firmly against the bottom (Beanlands, Can. Entomol. 98:412-414, 1966). A pair of beetles was introduced into each petri dish. Dishes were kept at room temperature, and gallery length, number of eggs, number of larvae, and mortality of eggs and larvae were recorded periodically.

In preliminary tests, the following compounds at various does up to 100 μg/insect were used by topical application to parent adults: (1) 4-ethylphenyl-6,7-epoxygeranyl ether (Pallos et al., Nature 232:486-487, 1971); (2) nonepoxy form of #1; (3) methylenedioxyphenyl-6,7-epoxygeranyl ether; (4) ethyl branches form of #3 at carbon 7 in the chain; (5) p-chlorophenyl-7-ethyl-6,7-epoxygeranyl ether (Bowers, Science 164: 323-325, 1969); (6) methyl ester of cis-dihydro(+)-todomatuic acid. The last compound occurs as free acid in some Douglas-fir trees and has shown morphogenetic activity against *Tenebrio molitor L.* (Rogers et al. Can. J. Chem. 52:1192-1199, 1974); (7) ZR-512; (8) ZR-515; (9) ZR-619; (10) ZR-777.

Only ZR-515 reduced hatching and affected larval development; consequently, this material only was used in further experiments, and was applied as follows: to the abdominal venter of parent adults at a rate of 50 μg/insect, or to the phloem as a 0.2% water emulsion to drip point. Untreated adults on phloem sprayed with water were used as controls. The dishes were examined every 2-3 days to record gallery length, numbers of eggs and larvae, and larval mortality.

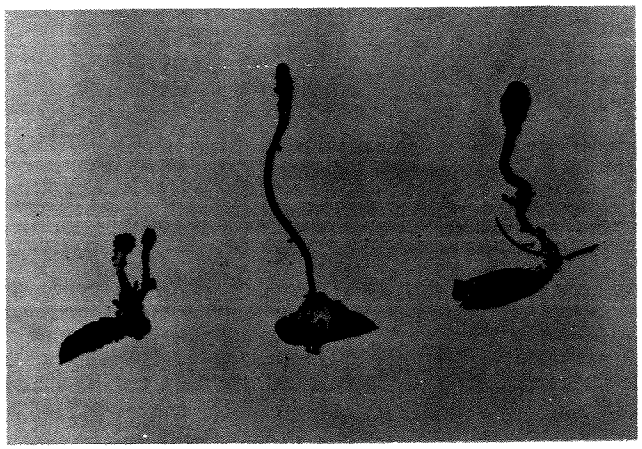
The data (Table 1) were subjected to the t-test. Application of ZR-515 slightly increased egg production in beetles but the difference was not significant ($p > 0.1$). Gallery lengths showed no noticeable differences. Application of IGR to the phloem reduced the percentage hatch significantly ($p > 0.05$). However, topical treatment was not effective ($p > 0.1$). The exposure of the larvae to the treated phloem produced significantly high larval mortality ($p > 0.01$), compared with the check and topical treatment. Thus, the results reveal that the IGR is not transmitted to the egg and larval stages by the adults, which degrade it promptly, but that it must be continually available to the progeny to produce its effect. The IGR as used here appears to cause larval mortality, perhaps by blocking larval maturation. (See page 5).

This report confirms Slama's contention (*op. cit.*, 1971) that bark beetles are generally insensitive to several IGR compounds. However, ZR-515 produced characteristic IGR-type results on Douglas-fir beetle progeny. As stated, ZR-515 must be applied to the host phloem to be effective and, since it is not systemic in action, its consideration for field use for bark beetle control depends upon the development of practical means of delivering it to the phloem.—A. Ibaraki and T. S. Sahota, Pacifiic Forest Research Centre, Victoria, B.C.

Preliminary Tests with a Fungus to Control Insect Defoliators.—The fungus *Cordyceps militaris* (L.) Link, a natural control agent of the green-striped forest looper [*Melanolophia imitata* Wlk.], kills the insect in the pupal stage, thereby reducing its population. However, significant population control does not usually occur until the insect host, western hemlock [*Tsuga heterophylla* (Raf.) Sarg.], is severely defoliated. The possibility of artificially increasing parasitism with this fungus by spraying infestations of feeding late-instar loopers with a formulation of homogenized cultures was tested in the summer of 1970 in northern Vancouver Island, during a heavy infestation.

Mass cultures of *C. militaris* were made on 3% malt broth and incubated for 5-6 weeks at room temperature (Funk, Bi-mon. Res. Notes 29:25, 1973). The cultures were obtained from ascospores produced on a naturally-infected pupa of *Melanolophia.* Immediately before spraying, each fungus mat, consisting of hyphae and conidia, was homogenized in a Waring blender for 10 seconds and diluted with tap water (one mat from 500 ml broth was diluted to 2 litres). A mist blower was used to blow the suspension onto the infested trees; a check area was sprayed with water.

Four weeks after spraying, most loopers had pupated and 100% of 100 pupae dug from the duff were infected, as shown by the presence of fruiting bodies of *C. militaris* (Figure 1).



A few larvae, beaten from the foliage and caught on a sheet, were sluggish, discolored and partially necrotic. In the check area, only 30% of 62 pupae were infected. Sixty percent of a sample of 100 loopers, removed from the test area before spraying, pupated.

Similar tests were made on other species of Lepidoptera in succeeding years: the western hemlock looper [*Lambdina fiscellaria fiscellaria* Guen.] at Allouette Lake in 1971; the false hemlock looper [*Nepytia freemani* Monroe] at Port Renfrew in 1972, and the black-headed budworm [*Acleris gloverana* Powell] at Port Renfrew in 1972. In these tests, there was no indication of infection nor any reduction in numbers that could be attributed to the fungus.

The success in the infestation of *Melanolophia* on northern Vancouver Island was probably due to a cool, moist microclimate and the burrowing habit of the larvae prior to pupation in the duff, which would favor survival and growth of *C. Militaris.* Further work is recommended in areas where favorable conditions exist.—S. Ilnytzky and A. Funk, Pacific Forest Research Centre, Victoria, B.C.

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Effect of Insect Growth Regulators on the Survival of Douglas-fir Beetle Progeny (cont'd.)

TABLE 1

Effect of ZR-515 on brood production and brood survival of Douglas-fir beetles

Treatment	Replicates	Gallery length (mm)		Eggs/gallery		% Egg mortality	Larvae/gallery		% Larval mortality
		mean	S.E.	mean	S.E.		mean	S.E.	
Check	10	224	30.9	27.8	4.3	27.0	20.3	3.2	28.6
Topical treatment of adults	11	201	32.1	30.3	4.4	36.0	19.4	4.7	29.9
Phloem sprayed	13	213	27.9	33.8	4.1	49.2	17.5	2.3	63.4

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