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bi-monthly research notes

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BOTANY

Phragmoid Cells of Leaf and Twig Scars on Northwest Poplar (*Populus X deltoides* Bartr. cv. 'Northwest').—Normal poplar leaf and twig drop follows the occurrence of the first frost in the late summer when night temperatures drop to -5°C. A second twig drop may occur during the next growing season after a spring frost affects foliated shoots. The time of the leaf drop and the number of days required to bring about defoliation by frost fluctuate from year to year. Poplar stands will retain discolored leaves in the autumn if there is a rapid freeze of leaf and bark tissues. Scar tissues form in the current year after early autumn leaf and twig drop during the warm daytime temperatures required for tissue formation. These tissues do not form readily if leaf drop is late. Leaf and twig drop and scar tissue formation starts with abscission, which involves meristematic activity and rapid dilation of isodiametric cells at the base of the leaf stalk and twig. The newly formed scar tissue has a phragmoid cell that is described here for the first time.

Several Northwest poplar trees obtained from the Provincial tree nursery at Oliver, Alta. were potted in the greenhouse during the autumn of 1973. They leafed out in December and were maintained until April 1974. Abscission was induced in 24 leaves in February by freeze-killing the petiole 5 mm above the point of attachment to the twig by applying 5s freon flow from a 1-mm nozzle attached to a pressurized container. Care was taken not to kill the basal part of the petiole or the bark to which it was attached as this would prevent abscission tissues from forming. Four leaf samples were checked during the first 2 days to ascertain the number of days required for meristematic tissues to develop at the base of the petiole. The leaves began to drop on the third day and sampling was discontinued. Six-week-old leaf scars were excised, cooked in 3% sodium bisulfite, rinsed, macerated, and observed under a light microscope.

Twig scars of Northwest poplar formed after a frost in September 1973 were obtained in January 1974 from a cultivated local tree. Samples were cooked and pulped in 1:1 mixture of acetic acid and 15% hydrogen peroxide. Pulped tissues from the leaf and twig scars were stained by adding 1% aqueous solution of chlorazol black at 107°C, rinsed several times, and mounted in Aman's lactophenol.

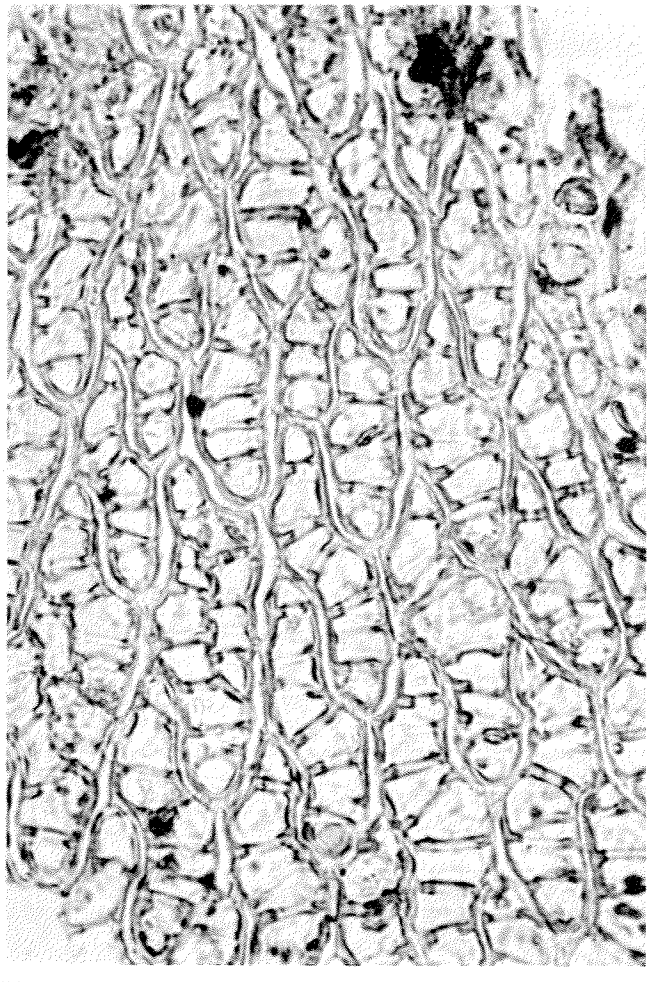

Figure 1.

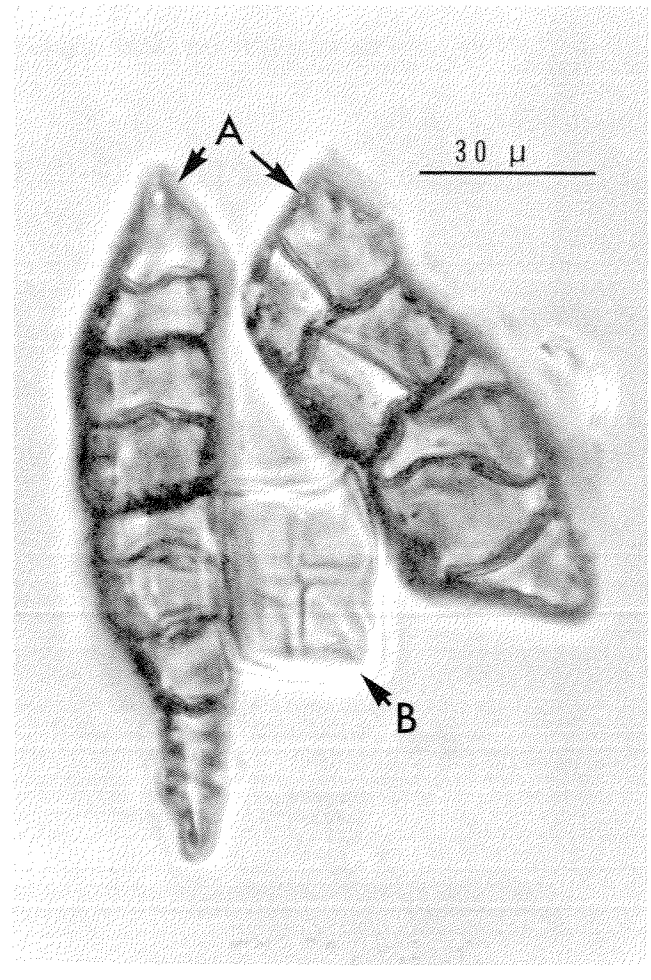

Figure 2.

The corky tissues of leaf and twig scars were heterocellular. They were composed of expanded fusiform cells arranged in irregular rows and contained a band or groups of elongate phragmoid cells (Fig. 1). Isolated phragmoid cells were heterogeneous, with or without scalariform pitted walls (Fig. 2A). The cell walls varied in thickness within and between these multicellular phragmoid cells. The most abundant cell within corky tissue was the angular, isodiametric thin-walled cell (Fig. 2B), and the least abundant was the sclereid-like cell (Zalasky, Inf. Rep. NOR-X-48, 1972).

The lumen of each fusiform phragmoid cell is packed with unequal angular cells arranged longitudinally, and occasionally horizontal intercallary cells may be present. This phenomenon is common in the fusiform initials of the cambium, glandular trichomes of certain plants, and the suspenor cell of certain embryos. The variations of phragmoid cells are probably due to the effects of internal division within the confines of the original cell wall.—H. Zalasky, Northern Forest Research Centre, Edmonton, Alta.

ENTOMOLOGY

Mating Disruption of Tussock Moths by Atmospheric Permeation with Synthetic Sex Pheromone.—Sex pheromones show promise as an environmentally acceptable means of suppressing insect populations (various authors in Birch (ed.), Pheromones, North Holland Publ. Co., Amsterdam 1974). The most appealing technique appears to be the permeation of the local atmosphere of a pest with a level of sex pheromone sufficent to disrupt its mating ability. Presumably, the atmospheric pheromone habituates the males rendering them incapable of responding successfully to the small amount of pheromone released by the females with the net result that males are unable to locate females and mate with them. The sex pheromone of the Douglas fir tussock moth, [*Orgyia pseudotsugata*], has been identified as (Z)-6-heneicosen-11-one (Smith *et al.*, Science 188: 63-64, 1975) and is commercially available. Although this compound has not yet been reported from females of other tussock moth species, it sexually stimulates and attracts in the field both white-marked [*O. leucostigma*] and rusty [*O. antiqua*] tussock moths (unpublished data). Therefore, laboratory experiments were conducted to determine whether (Z)-6-heneicosen-11-one, hereafter referred to as ketone, has the potential to disrupt the mating ability of these two species which are currently pests in several localities in Canada.

Experiments were carried out in covered 3.6 *l* glass jars lined on the bottom with filter paper and gauze. The ketone, in hexane solution, was deposited in known amounts (0.1 to 100 μg) on 2x2.5 cm frosted glass plates (ends of histological slides). After the solvent had evaporated, the plates were placed in small petri dishes on the bottom of the jars, each of which contained six 1-day old male tussock moths. Controls consisted of similar jars with untreated glass plates. One hour after the introduction of the chemical, five newly-emerged virgin females were placed in each jar. The number of successful matings was indicated by a count of egg masses which are normally deposited 30-60 minutes after successful copulation. These were corroborated by a count of spent females. Evaluations were made 6 and 24 h after the introduction of the females. Tests with each quantity of ketone were replicated 3-6 times (2-4 jars per replicate). Other chemicals evaluated for comparison were (Z)-6-heneicosen-11-ol (supplied by J. Weatherston, I.P.R.I.), disparlure ((Z)-7,8-epoxy-2-methyloctadecane, the sex pheromone of the gypsy moth, like the tussock moths a lymantriid species), a related compound 2-methyl-(Z)-7-octadecene, and (Z)-7-dodecenyl acetate. Each of these compounds was tested only at the 100 μg dose.

The results of the test with the ketone against white-marked tussock moths are shown in Fig. 1. The mating success of the experimentals was significantly lower than the controls ($p < 0.05$, χ^2 test) for each level of ketone tested except the 0.1 μg dose after 24 h. The reduction in mating success appears to be linearly related to dosage of ketone on the substrate and presumably,

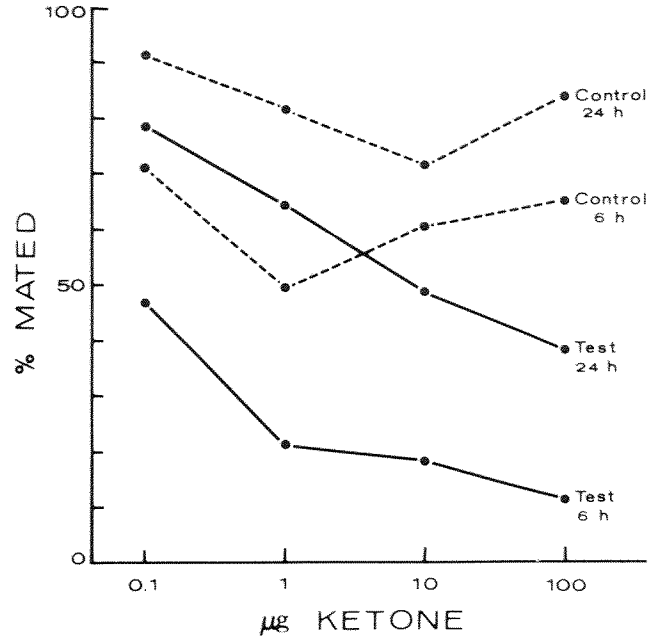

Figure 1. Mating success of *Orgyia leucostigma* moths exposed to various quantities of (Z)-6-heneicosen-11-one (ketone) compared to unexposed moths (controls) 6 and 24 h after introduction of females.

therefore, to the amount of ketone in the jar atmosphere. The percent mating reduction (difference in percent mating between experimentals and controls expressed as a percent of the percent mating controls) after 6 h ranged from 34% for the 0.1 μg dose to 82% for the 100 μg. These reductions in mating were greater than those obtained after 24 h when the reductions ranged from 14 to 55% over the same dosages. This decrease in mating reduction between 6 and 24 h may be due to the increased chances of a male accidentally coming into contact with a female where short range mating stimuli, such as visual and tactile cues, may take over from pheromones and lead to copulation. Undoubtedly the unnaturally high moth density in the jars was the reason that mating was not completely eliminated by any of the treatments. When the experiment was repeated with the rusty tussock moth, mating reduction with 100 μg of ketone was 70% after 6 h and 61% after 24 h ($p < 0.01$ in both cases).

Each of the four other chemicals tested against white-marked tussock moth males (Table 1) had some effect on the mating success of this species but only disparlure caused a statistically significant reduction in mating after 24 h. It is significant that in electroantennogram (EAG) tests, which measure the responsiveness of moth antennae to olfactory stimuli (Grant, J. Econ. Entomol. 64:315-316, 1971; Grant, Bi-mon. Res. Notes 31:19, 1975), disparlure was far more stimulating to male antennae of white-marked tussock moths than any of the other

TABLE 1

Mating reduction of *Orgyia leucostigma* moths caused by atmospheric permeation with various chemicals (100 μg on glass substrate)

Compound	% Mating Reduction 6h	24h
(Z)-6-heneicosen-11-one	82*	55*
(Z)-6-heneicosen-11-ol	33*	12
(Z)-7,8-epoxy-2-methyloctadecane	60*	32*
2-methyl-(Z)-7-octadecene	24	14
(Z)-7-dodecenyl acetate	15	16

* χ^2 significant, $p < 0.05$.

compounds except the ketone. The order of EAG stimulating effectiveness was ketone >> disparlure > (Z)-6-heneicosen-11-ol > 2-methyl-(Z)-7-octadecene > (Z)-7-dodecenyl acetate, the same order of their effectiveness in reducing mating success after 6 h. This suggests that the various chemicals mediate their effect on mating through the olfactory nervous system and not by acting as nonspecific irritants.

The results of these experiments indicate that (Z)-6-heneicosen-11-one could be used to disrupt mating of both the white-marked and rusty tussock moth provided the atmospheric concentration was high enough and the population density relatively low. A small scale field evaluation with this compound is warranted.—G. G. Grant and D. Frech, Insect Pathology Research Institute, Sault Ste. Marie, Ont.

Application of Dimilin Effectively Controls Forest Tent Caterpillar Populations and Affords Foliage Protection.—It was earlier reported that the insect growth regulator Dimilin (PH 60-40), a substituted phenyl urea, is very effective in inducing lethal abnormalities in the forest tent caterpillar [*Malacosoma disstria* Hbn.] (Retnakaran and Smith, Bi-Monthly Res. Notes 32(1) 1976).

Laboratory tests using artificial diet (Grisdale, Can. Entomol. 102:1111-1117, 1970) coated with low concentrations of wettable powder suspension of Dimilin (25% a.i.) showed that first-instar larvae were adversely affected (Table 1). Feeding preference tests (10 replicates) using diet pellets coated with Dimilin showed that larvae fed on treated and untreated diets alike indicating that they did not recognize treated from untreated diet. Moreover they tended to aggregate as much on treated as untreated pellets. It was expected therefore, that in the field the larvae would not actively seek foliage not contaminated with Dimilin.

Two 1/2 acre plots (0.2 ha) adjacent to each other near Rutter, Ont. were selected for the field test. Over 90% of the trees in the plots were trembling aspen [*Populus tremuloides* Michx.]. Ten trees from each plot were cut down during the first week in May and the total number of current year's egg bands were collected. Eggs from about 10% of the egg bands from each tree were counted and allowed to hatch to determine the average number of eggs per band and the average percent hatch. From these data, the total number of viable eggs per tree were calculated (Table 2).

A 1% suspension (active ingredient) of Dimilin was mixed in water and sprayed to run off with a back-pack power sprayer (GAF mist blower, Holland) on all the aspen trees in the treatment plot when the larvae were in the first and second instars. Post-spray sampling was done during the last week in May when most of the insects were in the last larval instar. Defoliation was not detectable in the treatment plot, and no larvae could be found either on the sampled trees or in the underbrush around the trees (Fig. 1a and Table 3). Larvae were present on the destructively sampled aspen trees in the control plot, and a substantial population was also present in the underbrush around the trees (Table 3). The trees were almost totally defoliated (Fig. 1b). The 560 larvae collected were reared in the laboratory and checked for pathogens and parasites. Of the 32 that died 2 were found to be infected with virus indicating little effect of *Entomophthora*, virus, or parasites.

TABLE 1

Effect of Dimilin on first instar larvae of forest tent caterpillar. The observations were made 8 days after treatment.

Treatment (mg Dimilin in 20 l diet cup)	No. of larvae treated (10 larvae/cup)	% Mortality	Developmental stage of those alive (%)			$\bar{X}$ weight/larva (mg)	Feeding activity
			First instar	Second instar	Third instar		
0.000(control)	50	14	0	77	9	5.55	good
0.750	50	54	32	10	4	1.22	poor
0.075	50	68*	30	2	0	1.18	poor

*Many of the larvae fed on the surface and hence the higher mortality.

TABLE 2

Pre-spray samples of forest tent caterpillar.

Plot	Sample values	No. trees sampled	Tree height (M)	Total no. egg bands	$\bar{X}$ no. eggs/tree	Total no. eggs/tree	$\bar{X}$ % hatch	Total no. viable eggs/tree
Control	$\bar{X}$	10	7.38	45.8	120	6828	36.5	2927
	Range	-	6.3-8.7	12-118	52-202	676-23836	6.6-62.0	134-10583
Treatment	$\bar{X}$	10	6.6	36.5	133	4939	31.1	1889
	Range	-	4.8-9.0	3-116	84-228	252-10857	12.0-61.4	86-6666

TABLE 3

Post-spray samples of forest tent caterpillar.

Plot	Sample values	No. trees sampled	Tree height (M)	No. larvae/tree	Hatched egg bands/tree	Defoliation	Larvae in underbrush around each tree
Control	$\bar{X}$	10	5.5	56.0	> 30	Total	Numerous
	Range		3.6-7.5	10-118	all > 30	All Total	All Numerous
Treatment	$\bar{X}$	10	6.1	0	> 30	Nil	Nil
	Range		3.6-9.0	0	all > 30	All nil	All nil

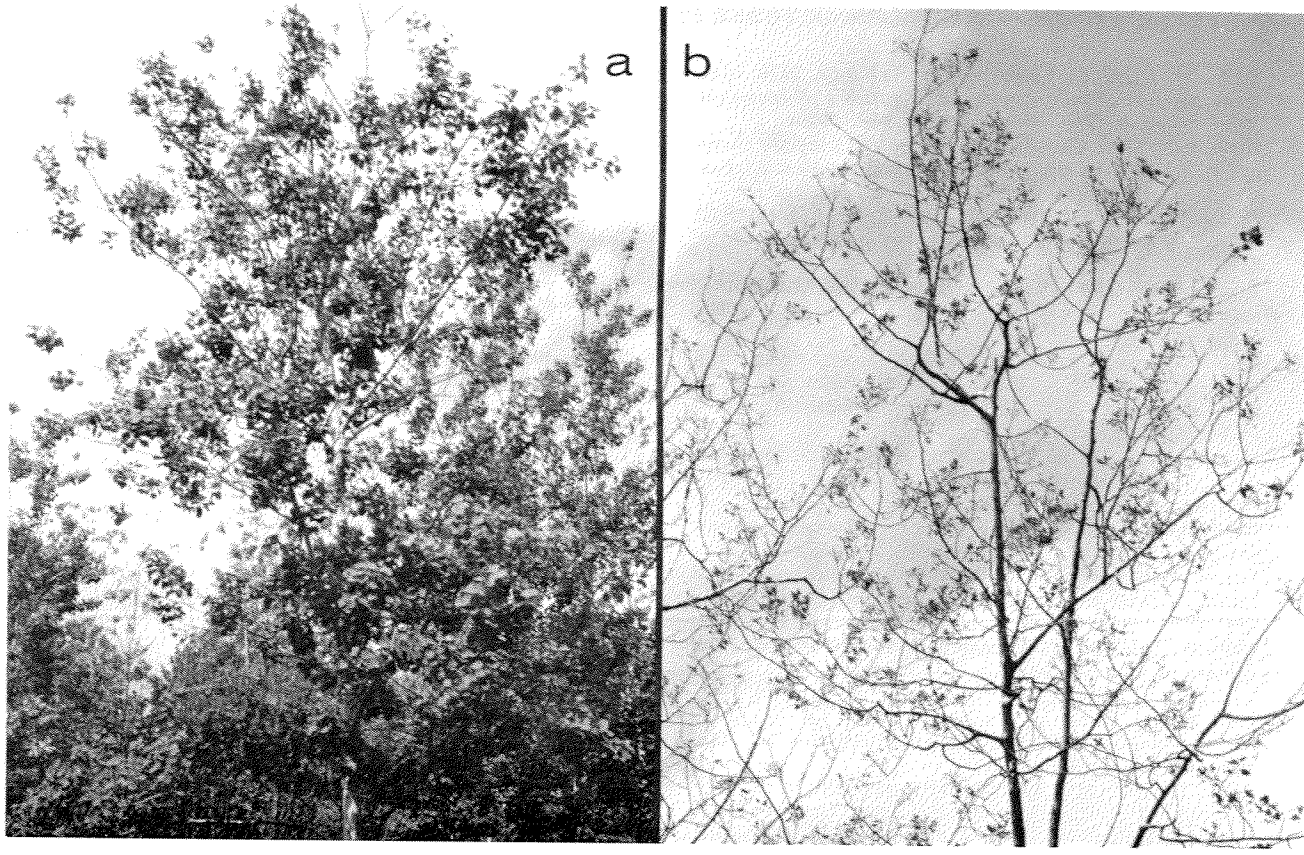

Figure 1. Effect of Dimilin on foliage protection against forest tent caterpillar. a) Treated with Dimilin b) untreated.

The spectacular control achieved with Dimilin together with the compound being an ecologically acceptable insect growth regulator warrant a larger field test next year perhaps with a helicopter equipped with boom and nozzle.—Arthur Retnakaran, Larry Smith and Bill Tomkins, Insect Pathology Research Institute, Sault Ste. Marie, Ont.

Further Calculations Relevant to Field Application of *Bacillus thuringiensis*. The degree of control obtained by aerial spraying of *Bacillus thuringiensis* against spruce budworm, [*Choristoneura fumiferana*], has been highly variable. Recently, (Bi-Monthly Res. Notes in press) we reported some calculations indicating that at least some of this variability might be due to the low dose of active material reaching the target site: extensive feeding was required for an LD_{50} dose to be consumed. Here we report further calculations suggesting that a very large loss of active ingredient occurs between mixing and impact on the target site.

The volume of a 100μ diameter droplet is 527,000 μ^3, which is equivalent to 1.9 million 100μ droplets/cc. A spray suspension containing 8 BIU/0.5 gal would contain 4.35 million IU/cc. Since each IU represents 2,000 crystals and 2,000 spores and there are 1.9 million droplets/cc then each 100μ droplet contains 2.28 IU or 4,580 crystals.

This value of IU/drop is true for the liquid suspension in the spray tank: each 100μ diameter unit of the suspension contains this number of IU. Presumably each 100μ droplet of spray suspension should also contain this number of IU as it leaves the spray nozzle. Nevertheless, Morris' data presented at the Symposium on Microbial Control of Spruce Budworm in Montreal 1976 indicates that 100μ diameter drops near the forest floor contained only 144 crystals or 0.07 IU; only 3% of the expected value. It appears that 97% of the active material is lost between mixing and impact at the target site. Evaporation during air-fall should concentrate particles and tracking dye leading to an effect opposite to that observed. Errors introduced into these calculations by factors such as spreading on impact might mitigate the severity of the loss of active material somewhat but not enough to invalidate the finding that large losses of active ingredient occur during application.

In our previous communication we reported the LD_{50} dose for a VIth instar spruce budworm to be 0.6 IU and LD_{90} dose 3.5 IU. Each 100μ diameter unit of spray mix (8 BIU/0.5 gal) contains 2.28 IU, almost 4X the LD_{50} dose although not yet approaching the LD_{90}. A VIth instar budworm larva would need to eat only two 100μ diameter drops or (at 30 drops/cm^2 of foliage) 0.06 cm^2 of foliage to get a lethal dose. If the active ingredient in the spray mix were reaching the canopy one could reasonably expect good control. The apparent loss between mixing tank and impact site may well be responsible for the low effective dose on the foliage reported earlier.

We are presently testing these calculations experimentally.—Paul G. Fast, Insect Pathology Research Institute, Sault Ste. Marie, Ont.

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