

bi-monthly research notes

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SOILS AND FERTILIZER

Growth Response to Aerial Forest Fertilization. — In comparison with small-scale experimental forest fertilization, the direct measurement of growth responses to large-scale operational forest fertilization is costly and time-consuming because it requires the use of intensive sampling techniques. On large tracts of forest land, responses to fertilization may be masked or obscured by the natural heterogeneity of the microclimate, topography, drainage, vegetation, and soils of the area. Response to aerial forest fertilization operations has been assessed (Mitchell and Kellogg, *Can. J. Forest Res.* 2:95-97, 1972; Windsor and Reines, *J. For.* 71:659-661, 1973) but has provided poor growth estimates for the entire forest since the assessments were confined to single plots (30-100 trees per plot) in fertilized and unfertilized areas. In this study, we attempted to obtain a more precise measure of the growth response to operational aerial fertilization by using a multiple-plot sampling technique in which fertilized and unfertilized plots were paired by similar pretreatment stand criteria.

Fertilization was undertaken by Scott Paper Company Ltd. on a young stand in central Nova Scotia that contained predominantly 10- to 20-year-old red and white spruce and balsam fir at an average stocking of about 23,000 stems/ha. The area is mapped as a Shulie series, a gravelly sandy loam soil derived from glacial till overlying sandstone bedrock (Wickhard and Smith, *N.S. Soil Surv. Rep.* 3, 1948). The terrain is gently sloping and affords good drainage. The area was marked into four adjacent strips (340 x 2 800 m), about 97 ha each. Two nonadjacent strips were fertilized; the other two were controls. Granular urea fertilizer was broadcast at the rate of 336 kg/ha (165 kg N/ha) by two fixed-wing aircraft, on 3, 5, and 6 October, 1970. Flight paths and fertilizer dissemination were monitored and controlled by observers at swath-width positions at opposite ends of each target strip. This helped to reduce the unevenness of spread associated with aerial applications of fertilizer (Armson, *Univ. Toronto For. Tech. Rep.* 11, 1972).

In November 1970, eighteen 0.004-ha circular plots were established at about 120 m intervals along the center line of each strip. Plot locations were staggered within 60 m of the center line to ensure placement on different flight paths. In each plot, all trees were marked with paint at breast height and tallied with calipers in 6 mm diameter classes. Ten randomly chosen dominant and codominant trees in each plot were tagged and measured with a height pole. The number of trees ranged from 42 to 78 per plot. Totals of 3,394 and 3,465 trees were measured in the fertilized and control areas respectively; the average tree heights were 3.66 m in the fertilized and 3.97 m in the control plots. In November 1974, four growing seasons after fertilizer treatment, the plots were remeasured by the same procedure. Mortality and ingrowth were determined from tallies of painted dead trees, and from unpainted live trees that had reached breast height.

One year after fertilization, current foliage samples were clipped from balsam fir and white spruce to assess N concentrations. Composite samples were analyzed from 10 trees per strip (5 at each end) for each species, for a total of 20 trees/species per treatment. Mean N concentrations were 0.42% higher in the fertilized area (1.60% N) than in the control (1.18% N), indicating that N fertilizer was absorbed by the trees.

After remeasurement in 1974, individual plot basal area (BA) was computed by totalling BA yield of each diameter class (i.e. class mark BA x no. of trees). Periodic BA increment was the difference in plot yields at the two measurements adjusted for ingrowth and mortality. Periodic height

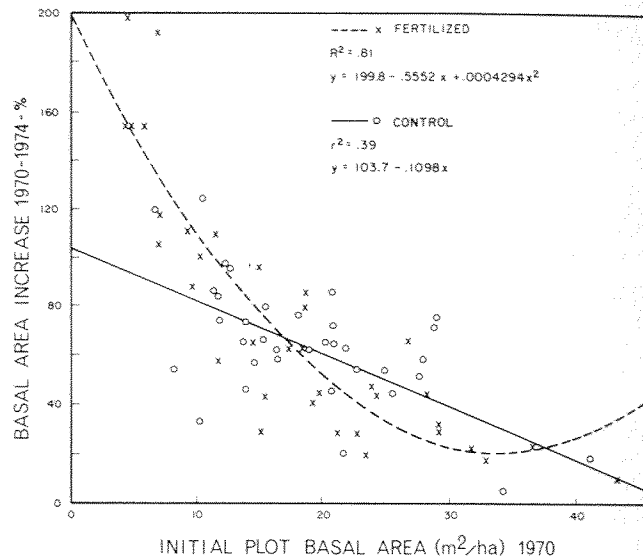


Figure 1. Relationship between periodic basal area increment and initial plot basal area.

increment was the difference in mean height of the 10 sample trees in each plot.

The systematic placement of plots in this area yielded a series of sample plots with a wide range in BA (Fig. 1). To ensure response comparisons between plots of similar BA, the initial plot basal areas in each strip were systematically ranked and then paired according to rank for each treatment. Both the absolute and the relative BA increment between paired plots were tested by ANOVA, and showed no significant differences between fertilized and unfertilized plots. However, noticeably larger BA increments were evident in fertilized plots of low BA (Fig. 1). Polynomial curves of degrees 1 to 3 showed no significant deviations from linearity in the control plots, whereas the fertilized plots had a significant quadratic component. Height response assessment involved similar pairing procedures, initial plot height being used instead of BA. Although ANOVA failed to detect significant height increment in absolute terms, relative height growth was significantly greater ($P > .05$), increasing from an average of 25.4% (control) to 28.6% (fertilized).

Despite the relatively large number of plots used in this operational assessment, the number, combined with the level of precision of measurement used, was insufficient to detect small differences in radial growth, given the stand variability encountered. To detect a BA increase of 10% with a 90% probability (at 0.05 significance level), it is estimated that 110 plots would be needed (Snedecor and Cochran, page 111 in *Statistical Methods*, Iowa State Univ. Press, 1972). Under these heterogeneous operational conditions, the assessment of height and diameter responses is apparently more sensitive in relative than in absolute terms. This may be the result of differing relative responses of plots at varying pretreatment stand conditions. — V.R. Timmer and R.A. Fisher, Maritimes Forest Research Centre, Fredericton, N.B.

TREE BIOLOGY

Anatomical Modifications in Xylem of Lodgepole Pine Container Seedlings Induced by TOK-E-25 (Nitrofen). — Auxin herbicide TOK-E-25 is a 2, 4-D derivative that affects a wide range of plant species during their active growing stage. Since nurserymen find handweeding almost prohibitive in closely spaced container seedlings, TOK-E-25 is commonly used for eradicating various broad-leaved weeds such as dandelions and Salicaceous germinants. However, actively growing conifer seedlings can also be very susceptible to such herbicides. This paper deals with anatomical effects of TOK-E-25 on the xylem of actively growing lodgepole pine container seedlings (*Pinus contorta* var. *latifolia* Engelm.) in the nursery.

The seedlings were developed to 5 weeks of age in the greenhouse and maintained outside for a full growing season. TOK-E-25 was applied at

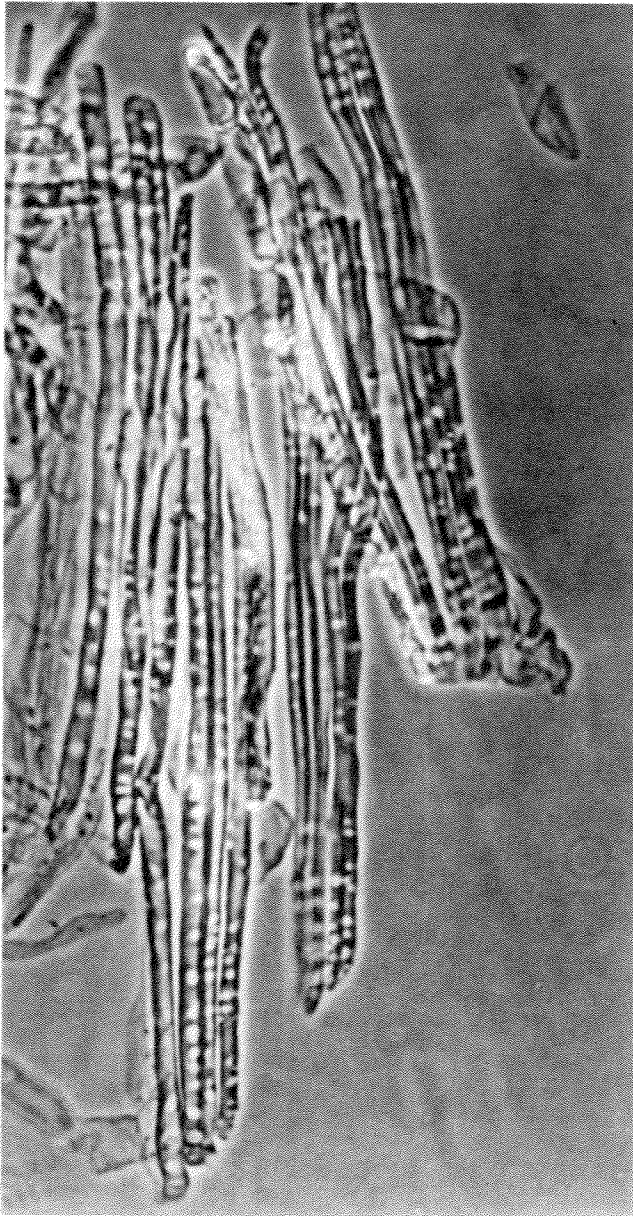


Figure 1. Five sets of daughter tracheids of lodgepole pine affected by TOK-E-25 show approximately the same stage of development without any sign of fiber tracheids.

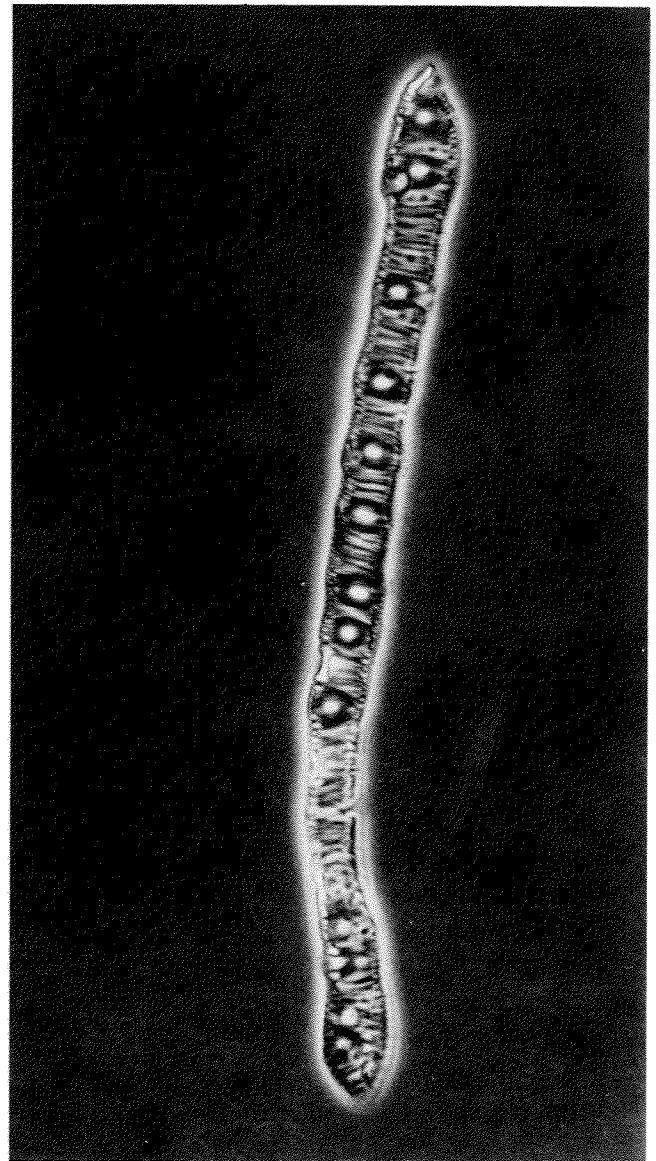


Figure 2. Spiral tracheid of lodgepole pine affected by TOK-E-25 has broad fibrils and bordered pits that are not normally found in primary tracheids.

weekly intervals during May, June, and July to control new weed germinants. First and second year's wood of lodgepole pine seedlings collected after July was prepared for anatomical study by macerating in a 1:1 mixture of glacial acetic acid and 15% hydrogen peroxide.

TOK-E-25 internal effects in seedlings can be readily verified by retarded development of affiliated daughter tracheids. The normal set of daughter tracheids matures as the new set is cut off by the mother cambium. In these seedlings, the first set in the rank of daughter tracheids was indistinguishable from the last set in the series. As many as five sets in the rank of daughter tracheids showed the same stage of development (Fig. 1); there were no mature tracheids or fiber tracheids in these sets. Other daughter tracheids matured to resemble vascular primary tracheids but were short with blunt ends and had double-bordered pits and broad spiral fibrils (Fig. 2) not unlike those of Douglas-fir. Others matured into fiber tracheids with fine spiral fibrils (Fig. 3). Fibril modifications in tracheids

caused by TOK-E-25 are diagnostically significant of external agents. Similar modifications were observed in frost rib tissues of Rosaceous species (Zalasky, *Can. J. Plant Sci.* 56:501-504, 1976) but not in fungus-induced swellings of pine (Zalasky, *Can. J. Bot.* 54:1586-1590, 1976). The fiber tracheids and tracheids in these pine seedlings were branched and curvate as in Fig. 3. They had a variety of shapes and sizes, as reported for frost-induced chimeras in cambial tissues (Zalasky, *Can. J. Bot.* 53:1888-1898, 1975).

The main difference between xylem affected by TOK-E-25 and frost- and fungus-affected xylem appear to be delayed maturation of daughter tracheids. The delay in xylem development means that diameter and height increment and phenology of the seedlings can be severely affected. Plants are slower-growing and have less chance of developing a normal bud (author's observations). Pine must develop secondary needles to produce a bud that yields a long shoot rather than just short shoots bearing needles. The presence of fibrils in tracheids is evidence that cambium affected by TOK-E-25 produced a higher proportion of primary than of secondary xylem.



Figure 3. Short spiral fiber tracheids of lodgepole pine affected by TOK-E-25 showing fine fibrils, curvation, and branching.

The herbicide 2, 4-D, from which TOK-E-25 is derived, has been shown to proliferate parenchyma, retard development of vascular strands, and inhibit root growth and primary needle expansion in 2- to 18-day-old pine seedlings (Wu et al., Can. J. Bot. 49:1737-1741, 1977). TOK-E-25 is a highly mobile compound in conifer tissues. Although its activity is suspended over the winter months, it persists in the cambium and xylem during the second growing season, presumably as breakdown products. — H. Zalasky, Northern Forest Research Centre, Edmonton, Alta.

PHYSIOLOGY

Low Molecular Weight RNA in Jack Pine (*Pinus banksiana* Lamb.) Seedlings. — A new class of ribonucleic acid (RNA) with low molecular weight has been detected in animal (HeLa) (Weinberg and Penman, J. Mol. Biol. 38:289-304, 1968) and bacterial (*Escherichia coli*) cells (Ikemura and Dahlberg, J. Biol. Chem. 248:5024-5032, 1973). This RNA has been termed smRNA. Its occurrence adds to the already recognized RNA types such as messenger, ribosomal, and transfer RNA's. Our report provides evidence for smRNA in jack pine. The new class of low molecular weight RNA has so far not been observed in gymnosperms. In animal cells Weinberg and Penman (1968) observed that the RNA fraction contained six discrete species ranging in size from 100 to 180 nucleotides (4-6S RNA). These components represented only 0.4% of the total RNA and were metabolically stable. Bases of at least four of the components were extensively methylated. Although the authors termed this fraction "the small molecular weight monodisperse nuclear RNA's,"

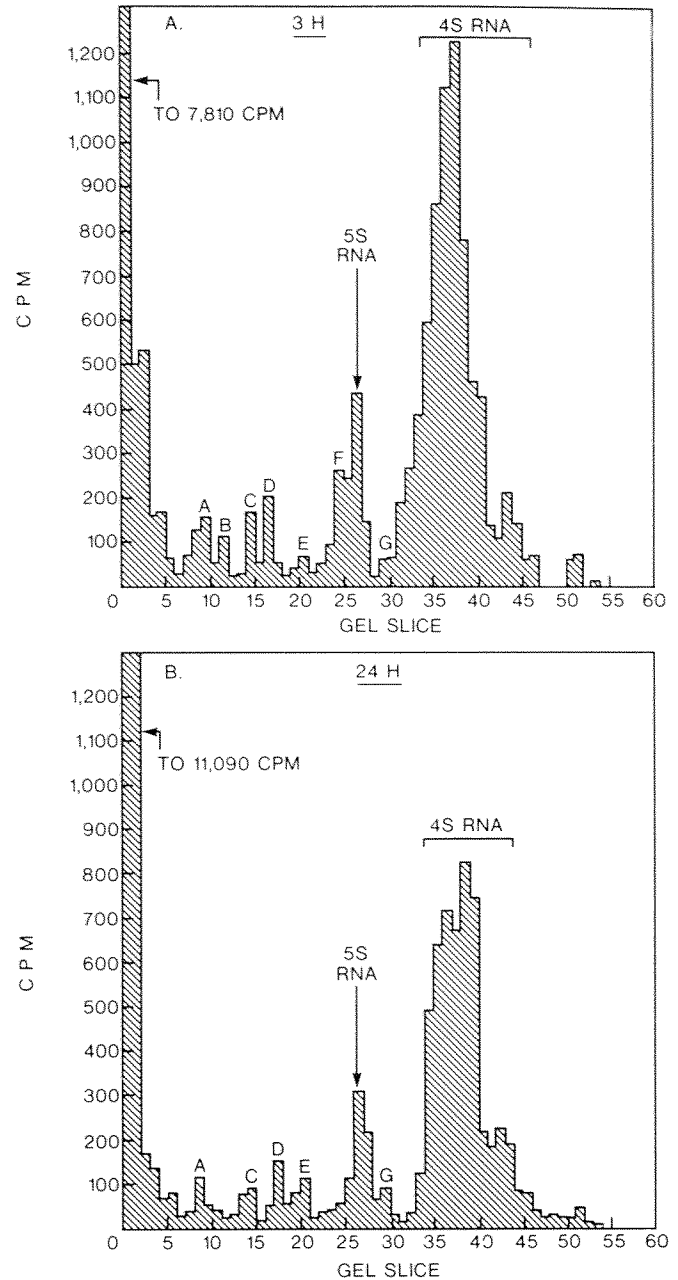


Figure 1. Distribution of radioactivity in jack pine RNA along a 10% polyacrylamide gel after a (A) 3-h and a (B) 24-h exposure to 50 μ Ci uracil-6- 3 H. SmRNA's are identified by radioactive peaks A to G. Migration of RNA is toward the anode at the far right.

other studies have shown that these components are also present in the cytoplasm (Frederiksen and Hellung-Larsen, FEBS Lett. 58:374-378, 1975). In the latter study, unstable precursors to the smRNA's and four stable low molecular weight RNA's were identified.

Jack pine seeds were obtained from the Petawawa Plains, Ont. Seeds with germination greater than 95% were surface-sterilized with 5% calcium hypochlorite, washed, and germinated under constant light not exceeding 6.2, 5.5, and 2.7 μ W/cm 2 /nm in the blue, red, and far-red respectively. Six-day-old seedlings were exposed to 50 μ Ci of uracil-6- 3 H (specific activity 8.9 Ci/m mol). Duplicate samples of seedlings were taken after 3 and 24 h of exposure. Seedlings were washed in distilled water and extracted for total RNA (Pitel and Durzan, Can. J. Bot. 53:673-686, 1975).

After electrophoresis of RNA (Durzan et al., Can. J. Forest Res. 2:206-216, 1972) the polyacrylamide gels were sliced into 1 mm segments and placed in vials, and radioactivity was determined in Bray's solution by liquid scintillation spectrometry (Loening, Annu. Rev. Plant Physiol. 19:37-70, 1968).

Fig. 1(A) shows 5S ribosomal RNA, 4S transfer RNA and seven discrete small molecular weight RNA components (labelled A to G) present after a 3-h incubation with uracil-6-³H. By comparison with *E. coli* RNA, these components range from 4S to 8S in sedimentation behavior. Similar peaks of radioactivity for smRNA of animal cells have also been observed (Weinberg and Penman, 1968). In jack pine five of these components were detected after a prolonged incubation of 24 h (Fig. 1[B]) while the remaining two (B and F) were not detected and thus were probably not as stable as the other five.

Criteria for the components in jack pine (labelled A to G) that support the view that they are smRNA's typical of those found in animals are: (1) they are composed of RNA (treatment with ribonuclease removes all radioactive bands from the gel); (2) they have a low molecular weight range that does not exceed 4S to 8S RNA; (3) they represent a small (less than 1%) fraction of the total RNA; (4) electrophoretic mobilities of components are reproducible and consistent with reported properties of smRNA — i.e. this fraction is not an artifact of electrophoresis or RNA degradation; (5) most of the smRNA's were stable as judged after a prolonged 24-h incubation with uracil-³H followed by a "chase" with cold uracil (two components [B and F], not present after the 24-h incubation [Fig. 1(B)], are postulated to represent precursors either for 4S transfer RNA and 5S RNA or for the smRNA's [Frederiksen and Hellung-Larsen, 1975]).

The overall significance of the smRNA is unknown. It has been proposed that some smRNA's may be required for protein synthesis (Hellung-Larsen et al., Exp. Cell Res. 85:1-7, 1974) and for DNA replication and folding (Ikemura and Dahlberg, 1973).

In summary, since our evidence suggests that the RNA complement of jack pine contains smRNA's, the range of smRNA would be extended to gymnosperms. — J.A. Pitel, Petawawa Forest Experiment Station, Chalk River, Ont., and D.J. Durzan, Institute of Paper Chemistry, Appleton, Wis.

INSECT PATHOLOGY

Microsporidian Infection in Spruce Budworm (*Choristoneura fumiferana*) 1 and 2 Years after Application. — In 1975 a microsporidian, *Nosema fumiferanae* (Thom.), was tested against the spruce budworm on Manitoulin Island, Ont. The levels of infection for the year of spraying and the following year have been reported (Wilson and Kaupp, Bi-Mon. Res. Notes 32:32, 1976). In June 1977, samples of spruce budworm larvae were taken from the same spruce (*Picea glauca* [Moench] Voss) trees. Briefly the percent levels of infection were as follows: 1975 (year of application), 53.0; 1976, 52.4; 1977, 53.8. The checks were 23.0, 39.0, and 48.0 respectively. Levels of *N. fumiferanae* have remained similar in the treated trees, while natural infection in the checks has risen to almost the same levels. The data suggest that, in this particular population, the levels of infection were advanced 2 to 3 years by the application of microsporidian spores.

During the summer of 1976 two microsporidians, *N. fumiferanae* and *Pleistophora schubergi* Zwölfer, were tested against the spruce budworm approximately 15 km north of Thessalon, Ont., in Rose Township. Experimental details and results for the first year of spraying have already been reported (Wilson and Kaupp, Can. For. Serv. Inf. Rep. IP-X-15, 1976). In June 1977, samples of spruce budworm larvae were taken from some of the trees that were sprayed in 1976. Smears were prepared from the larvae and examined microscopically for the presence of microsporidian spores. The results are shown in Table 1.

There was a decrease in the levels of *N. fumiferanae* in larvae collected from balsam fir (*Abies balsamea* [L.] Mill.) the year after application. However the incidence was still significantly higher than the checks. Infection of *N. fumiferanae* in larvae collected from treated white spruce remained virtually the same for both years, but there was a substantial increase in the infection levels for insects in the check area.

The carry-over of *P. schubergi* was low, following the high infection levels in the year of spraying. This may have been due, in part, to mortality

TABLE 1

Incidence of *Nosema fumiferanae* and *Pleistophora schubergi* in living spruce budworm larvae collected after application¹

Trees	Treatment	Date sampled	No. insects examined	Incidence (%) of	
				<i>N. fumiferanae</i>	<i>P. schubergi</i>
White spruce	Nf	28 June, 1976	285	54.7*	—
Balsam fir	Nf	10 June, 1977	112	53.4	—
White spruce	Ps	28 June, 1976	48	72.9*	—
Balsam fir	Ps	10 June, 1977	54	53.0*	—
White spruce	check	28 June, 1976	32	—	48.6
Balsam fir	check	10 June, 1977	32	—	3.1
White spruce	check	28 June, 1976	63	—	95.2
Balsam fir	check	10 June, 1977	24	—	0
White spruce	check	28 June, 1976	55	25.0	0
Balsam fir	check	10 June, 1977	72	40.0	0
White spruce	check	28 June, 1976	67	38.8	0
Balsam fir	check	10 June, 1977	50	32.0	0

¹ Trees were sprayed on 3 and 4 June, 1976, with 5x10⁶ *N. fumiferanae* (Nf) and *P. schubergi* (Ps) spores per tree.

* Significantly different from the checks at the 5% level; t-test applied to percentages.

of infected insects. Population reduction studies were not performed, as our main purpose was to determine if these protozoans could be successfully introduced into a population of the spruce budworm. Collections will be made from the same areas in succeeding years to determine changes in the incidence of microsporidia. — G.G. Wilson, Forest Pest Management Institute, Sault Ste. Marie, Ont.

PATHOLOGY

A Source of Bright Light for the Stereomicroscope. — Forest pathologists and mycologists working with the stereomicroscope frequently need a powerful source of light to examine cankers, wounds, and deep crevices and cracks in the bark in order to pick out tiny fungal fruiting bodies that are often dark and inconspicuous. The microscope lamps sold for this purpose by scientific apparatus dealers have too weak and/or too small a beam of light and are not adequate for the work. To fill our needs, we designed and built a system yielding a wide, powerful beam that, after being experimented with for 2 years, has proven practically indispensable (Fig. 1). Minute fruiting bodies of organisms causing important diseases have been found on specimens examined without results 4 and 7 years before. The fairly simple device is based on a quartz-halogen lamp used in automobiles. The 12-V source required is a standard battery charger (6-12 V) worth about \$25. In addition to supplying the necessary direct current, the weight of the charger is sufficient to serve as a stand for the whole system.

Two pieces of aluminum sheet metal 5 mm thick were used to build the stand of the lamp (Fig. 2). The main piece, which serves as a crutch (C), is at first bent to form a 180° arc; a 7.5 cm flat portion is left at the larger end for attachment to the charger with four nylon bolts and screws like those used to secure license plates. The second aluminum piece, oblong-shaped, becomes a jib (J) of the stand, permitting more flexible adjustment of the beam to any point in the field of the microscope and allowing the base to be further away from the field of operation. The crutch and the jib are tied by a 9 mm screw bolt and a lock washer. At this point a washer cut from a thin sheet of teflon and placed between the two pieces (C and J) gives more flexibility to the coupling.

The quartz-halogen lamps are available with two types of fixtures. The first has a vertical adjustment on a screw nut (hinge type) and a horizontal (or circular) adjustment left to the securing screw nut; there also a teflon washer helps to give flexibility and facilitate setting up. The second type is different in that the lamp support is placed on a hemispherical joint, the attaching screw nut being used for both vertical and horizontal (circular) adjustment. For this last type of attachment, a large doorknob welded to the nut serves to loosen and tighten the adjusting screw when the direction of the beam is being changed.

The electric assembly is uncomplicated. The charger has two wires distributing the 12-V DC: a positive red and a negative black with terminal clamps, which must be removed. The red wire is then connected to the red wire of the lamp, and the black ground wire is attached to the crutch with a metal screw in a thread cut with a screw tap. As already stated, the base of

ENTOMOLOGY

A Method for Obtaining Mated Spruce Budworm. — During the past few years various techniques have been employed at the Maritimes Forest Research Centre to obtain mated female spruce budworm *Choristoneura fumiferana* [Clem.] moths for experimental purposes. The technique that gave the most consistent results is as follows. Pupae, one per vial, were exposed to a photoperiod of 17 h from 1600 h to 0900 h and to a temperature regime of 10-20-30°C based on a 24-h sine curve fluctuation. The temperature increased from 20°C at 0800 h to 30° at 1400 h.

Most of the moths emerged during the scotophase. They were kept under the same light regime as the pupae but at a constant 20°C. Females that emerged between 0800 h and 1600 were kept in the light at 20°C until 0830 the following day (i.e. the females were 20 ± 4 h old), when they were placed in a mating cage (30 x 30 x 45 cm high) with males that had emerged 1 day earlier (i.e. males were 44 ± 4 h old). Densities from 27 to 150 moths per cage have been used with no difference in mating success. Initially, a ratio of two males to one female was used but this was changed to a 1:1 ratio. The mating cage was examined 30 min after the start of the scotophase and each mating pair was removed and placed in a separate container for completion of mating. Mating success was consistently high, 95% or more of the females being mated within the first hour of the scotophase. — A.W. Thomas, Maritimes Forest Research Centre, Fredericton, N.B.

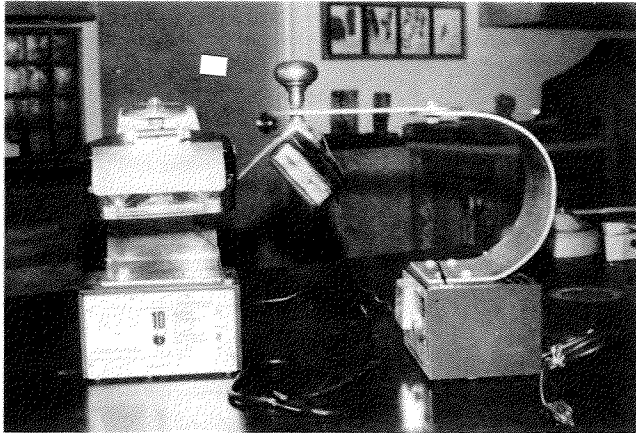


Figure 1. Two types of lamps: hinge type (left); hemisphere type (right).

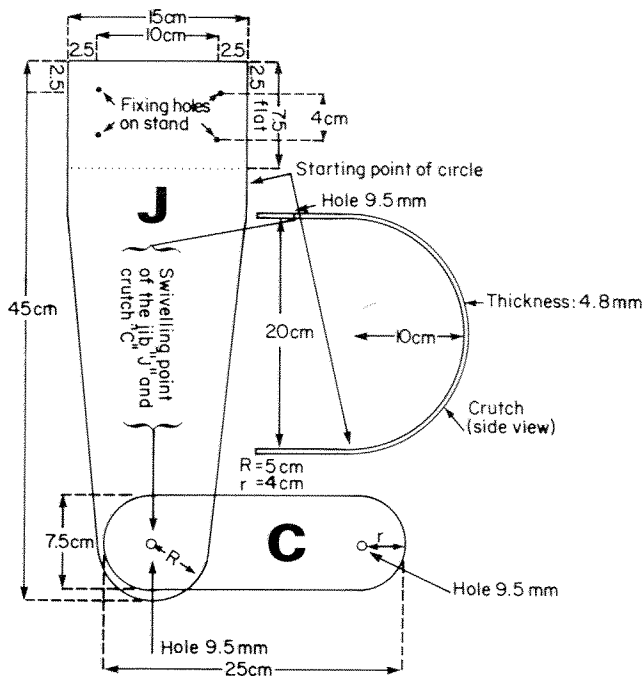


Figure 2. Design and cross section of the stand components (aluminum): crutch (C) (front view, left, and side-face view, right), after semicircular bending; extension jib (J) seen as designed and positioned on the crutch.

the crutch is attached with four insulating screw nuts to the box of the charger, from which it is well insulated by a piece of 1 mm rubber, or formica, arborite, or any other insulating material. A tumbler switch (sometimes supplied with the automobile lamp) is fastened on the charger box at the right front corner (on the plug wire side) and inserted in the source circuit (110-V AC). To do this, the red wire of the plug cable is cut (inside the box), and the two ends are lengthened and connected to the two terminals of the switch. A small piece of stainless steel or aluminum plate (about 15 cm x 7 cm) held to the frame of the lamp by two sheet-metal screws serves as an eyeshade or shield against the light, which is very bright and direct. The lamp is now complete and ready to use; it is powerful, being five to six times brighter than the best commercial ones. It can be assembled in less than 2 h once all components are at hand, and its cost does not exceed \$70. — R. Cauchon, Laurentian Forest Research Centre, Sainte-Foy, Que.

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