Environment Canada
Canadian Forestry Service

Environnement Canada
Service canadien des forêts

ISSN 0228-9989

canadian forestry service research notes

Viruses Infecting Five Species of Lepidoptera

Isolation and Culture of *Massospora levispora*

Fireblight of Wild Raspberry on Clear-cut Forest Areas

Timing and Duration Effects of Gibberellin and Fertilizer Treatment on Strobilus Production in Young Western Hemlock

VOL. 3, NO. 1, JANUARY–MARCH 1983

Canada

canadian forestry service
research
notes

A selection of notes on current research conducted by the Canadian Forestry Service and published under the authority of the Minister, Environment Canada. A French edition is published under the title of Revue de recherches du Service canadien des forêts. *It is the editorial policy of this publication that the names of members of review boards considering manuscripts for publication shall not be disclosed. Manuscripts submitted for review will be accepted for consideration only on this basis.*

INSECT PATHOLOGY

Isolation and Culture of *Massospora levispora*. — The fungus *Massospora levispora* Soper, a pathogen of the adult stage of the cicada *Okanagana rimosa* (Say), was discovered near Searchmont, Ont., where its host is periodically abundant (Soper, Can. J. Bot. 41:875–878, 1963).

Infected cicadas bearing conidia of the fungus were collected near Whitman Dam, Searchmont, in June 1971. The conidia were scraped from the conidial mass using a sterile needle and suspended in Grace's insect tissue culture medium (Grace, Nature 195:788–789, 1962), supplemented with 5% (v/v) fetal bovine serum (FBS), or in modified insect tissue culture medium (Yunker et al., Science 155:1565–1566, 1967). The conidia germinated within 24 h at ambient room temperature (22 ± 1°C) by means of a single germ tube about 5μm in diameter, often followed by the formation of one or two secondary germ tubes. In most cases, however, the protoplast did not divide, but migrated along the primary germ tube. Septa were formed at intervals along the germ tube behind the advancing protoplast. The germ tubes did not proliferate further, but on the modified medium after about 7 days incubation the protoplast was released into the medium, where it began to multiply vegetatively in a manner similar to that recorded earlier for *Entomophaga (=Entomophthora) egressa* MacLeod and Tyrrell (Tyrrell and MacLeod, J. Invertebr. Pathol. 12:755–760, 1972). The isolation experiments were repeated with conidia collected from the same area in June 1978, when it was found that a 50:50 (v/v) mixture of Grace's medium and FBS, or FBS alone, were also suitable media for isolation of the protoplast stage that appeared in the cultures after 8 days incubation. Once established in culture, the protoplasts can be routinely subcultured on Grace's medium plus 5% FBS.

The demonstration that *Massospora levispora* can exist and multiply as a protoplast thus brings to six the number of entomophthoraceous fungal genera in which one or more species has been shown to manifest this property, either in culture or *in vivo*. The other five are as follows: *Entomophaga* (Tyrrell, Exp. Mycol. 1:259–263, 1977); *Erynia, Triplosporium (=Neozygites)*, and *Entomophthora* (Butt et al., J. Gen. Microbiol. 127:417–421, 1981); and *Strongwellsea* (Humber, Ph.D. thesis, Univ. of Washington, 1975, quoted in Butt et al. 1981).

This information confirms an earlier suggestion (Tyrrell, 1977) that the phenomenon is more widespread than was suspected in entomogenous fungi. — D. Tyrrell and M.A. Welton, Forest Pest Management Institute, Sault Ste. Marie, Ont.

Viruses Infecting Five Species of Lepidoptera. — Pathogens occurring in wild insects are routinely identified in specimens obtained from survey samples. This report includes the description of viruses infecting five species of Lepidoptera; these are considered to be new records except where noted. The viruses were tentatively identified by microscopic examination of squash preparations of larvae using dark-field or phase contrast illumination. The identifications were confirmed by electron microscopic examination of material prepared following standard fixing and embedding procedures (Percy et al., Can. J. Zool. 58:2105–2115, 1980).

A sample of the chain-spotted geometer, *Cingilia catenaria* (Drury) (Geometridae), was collected in 1979 from larch in East Luther Township, southern Ontario. Several specimens were found to be infected with a multi-capsid nuclear polyhedrosis virus (MNPV) (Fig. 1). The presence of an NPV in populations of this insect has been previously reported from material examined in Quebec in 1973 (Martineau and Lavallee, Annu. Rep. Forest Insect and Dis. Surv., Can. For. Serv., p. 31–49, 1973).

A sample of the juniper looper, *Thera juniperata* L. (Geometridae), was collected in 1978 from red juniper in Sault Ste. Marie, Ont. A total of 23 larvae were examined; three were infected with a cytoplasmic polyhedrosis virus (CPV) (Fig. 2).

A sample of the cherry scallop shell moth, *Hydria prunivorata* Ferg. (Geometridae), was collected in 1981 from black cherry in South Walsingham Township, Simcoe District, Ont. A total of 31 larvae were examined; two were infected with a granulosis virus (GV) (Fig. 3).

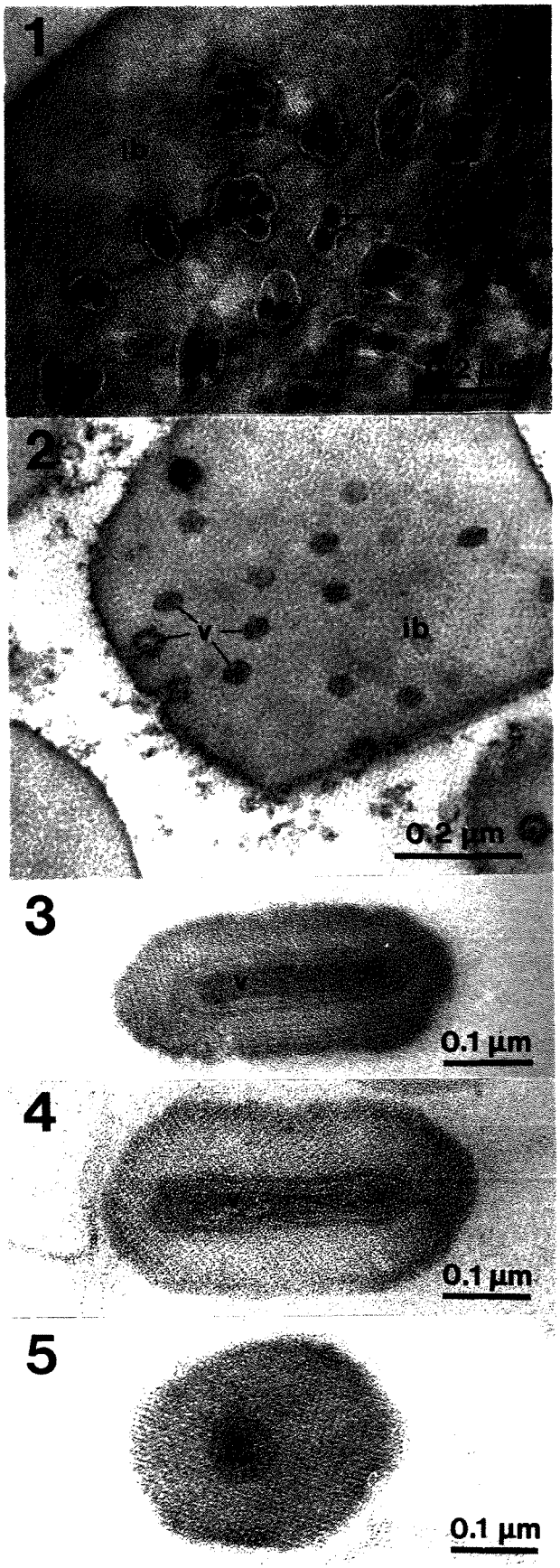

Figure 1. MNPV from *Cingilia catenaria.*

Figure 2. CPV from *Thera juniperata.*

Figure 3. GV from *Hydria prunivorata.*

Figure 4. GV from *Archippus packardianus.*

Figure 5. GV from *Rhyacionia buoliana.*

ib = inclusion body; v = virus particle.

Samples of the spring spruce needle moth, *Archippus packardianus* (Fern) (Tortricidae), were collected in 1953–1954 from Nipigon, Ont., and again in 1967–1968 from Portage La Prairie and Malonton, Man. Granulosis virus was tentatively identified from the first sample and confirmed in specimens obtained during 1967–1968 (Fig. 4).

A sample of the European pine shoot moth, *Rhyacionia buoliana* (Schiff.) (Olethreutidae), was collected in 1980 from Richmond, B.C. A total of 85 larvae were examined; 26 were infected with a granulosis virus (Fig. 5). This report is the first one of a virus occurring naturally in this species although it is known to be susceptible to the granulosis virus of the codling moth, *Laspeyresia pomonella* L. (Olethreutidae) (Huber and Dickler, J. Econ. Entomol. 70:557–561, 1977). — John Burke and Jean Percy, Forest Pest Management Institute, Sault Ste. Marie, Ont.

PATHOLOGY

Fireblight of Wild Raspberry on Clear-cut Forest Areas. — Fireblight of cultivated raspberry has been reported in the United States and the name *Erwinia amylovora* (Burr.) Winslow et al. f. sp. *rubi* was proposed for the causal organism (Starr et al., Phytopathology 41:915–919, 1951). In a search for diseases that were potentially effective in biological control of wild raspberry (*Rubus idaeus* L. var. *strigosus* [Michx.] Maxim), a species that interferes with the establishment and growth of forest trees, a similar disease was found on clear-cut forest areas in the Maritimes.

The disease appeared as extensive blackening of the juvenile stems and leaves of plants in localized patches. It was first observed on the Cape Breton Highlands of Nova Scotia in July 1979 during a period of wet weather. In 1980 it was not observed, but early in 1981, mild infections were found in the Green River Watershed of New Brunswick.

Cultures were readily obtained by incorporating small amounts of macerated tissue in nutrient dextrose agar. The bacterium grew readily on Miller-Schroth

medium, giving orange colonies characteristic of *E. amylovora* (Miller and Schroth, Phytopathology 62: 1175–1182, 1972). Electron micrographs showed flagellated, rod-shaped bodies similar to published photographs of *E. amylovora* (Van der Zwet and Keil, USDA Handbook No. 510, 1979).

Numerous inoculation attempts were made in the field and greenhouse by applying suspensions of bacterial cultures or freeze-dried bacteria in talc to juvenile plants. In the greenhouse, inoculated plants were left in a mist chamber for several days, and one series was subjected to cold treatments before and after inoculation. In the field, inoculations were performed at the beginning of a rainy period. In most cases, mild symptoms were observed 1 wk after inoculation, but severe symptoms, as found in Cape Breton in 1979, were not produced. Further studies on the physical requirements for survival of the bacterium and infection of plants are needed before this organism can be considered a potential biological control agent. In research of this type, consideration must also be given to the effects of pathogens on closely related cultivated plants, thus transfer of more pathogenic strains of the bacterium (if they exist) from other regions is inadvisable at this time. — R.E. Wall, Maritimes Forest Research Centre, Fredericton, N.B.

TREE PHYSIOLOGY AND ANATOMY

Timing and Duration Effects of Gibberellin and Fertilizer Treatment on Strobilus Production in Young Western Hemlock. — This investigation was conducted to identify the appropriate timing and duration of gibberellin-based treatments for induction of strobili in seedlings and rooted cuttings of western hemlock, *Tsuga heterophylla* (Raf.) Sarg. Production of male and female strobili may be induced reliably in juvenile plants of this species through applications of the gibberellin mixture $A_{4/7}$ and nitrate fertilizer. This method, based on the relative efficacy of less polar gibberellins compared to more polar forms such as A_3 (Pharis and Kuo, Can. J. Forest Res. 7:299–325, 1977) was developed through a series of field experiments (Ross et al., Can. J. Forest Res. 11:90–98, 1981), with further improvements achieved through imposition of water stress (Brix and Portlock, Can. J. Forest Res. 12:76–82, 1982) and increased temperature (Pollard and Portlock, Can. For. Serv. Res. Notes 1:21–22, 1981). However, environmental refinements have also increased the need to limit duration of treatments, because the costs of both gibberellin application and environmental control tend to deter the use of these treatments in operational breeding and seed production.

When applications of gibberellin were delayed until early July, the reproductive response of western hemlock seedlings was drastically reduced; furthermore, little benefit resulted from extending treatments from 6 to 12 wk when commenced in late May (Ross et al. 1981). The objectives of this experiment were to identify more clearly the period critical for maximum response and to determine the degree to which treatments could be shortened without reducing this response. Experimental materials were 3-yr seedlings and rooted cuttings. The experiment comprised a series of sequential or overlapping treatments applied in the spring of 1980, conducted in a heated greenhouse to enhance strobilus production.

Seedlings were reared in a peat and vermiculite mix in Styro-2 containers in a heated greenhouse, and were transplanted into 15-cm diameter plastic pots at the beginning of their 2nd yr. From transplanting to the beginning of the experiment, seedlings were grown in a shadehouse at the Pacific Forest Research Centre, Victoria, B.C. Cuttings, rooted in beds of sand, were similarly transplanted and reared in the shadehouse until their 3rd yr, when they were transferred to the greenhouse immediately before treatment. Each treatment contained 10 seedlings and 10 rooted cuttings, arranged in a randomized design of 5 blocks. Each block contained nine rows, with two seedlings and two cuttings in each row for each treatment. The nine rows represented eight treatments and an untreated control, and were temporarily separated from adjoining rows by plywood sheets during spray applications of gibberellin solution.

Treatments consisted of different periods of weekly application of $A_{4/7}$, combined with 10 g calcium nitrate applied every 2 wk to the soil surface. Dates of commencement ranged from 8 April to 1 July 1980, with durations from 4 to 16 wk. The full span of the experiment period was 8 April to 29 July 1980. Periods of each treatment are defined in Figure 1. Gibberellin $A_{4/7}$ was used at a concentration of 200 ppm by weight, dissolved in distilled water with a wetting agent (0.1% Aromox $C_{12}W$), and sprayed until all foliage was dripping wet. All plants were watered weekly. Greenhouse temperatures were maintained at a minimum of 24°C and increased up to 31°C in sunny periods. Photoperiods were natural.

Plants were returned to the shadehouse at the end of the final treatment period (29 July 1980) and were watered periodically until strobili were counted on emergence in April 1981.

Analysis of variance (Table 1) of male and female strobili produced in seedlings and cuttings (Fig. 1) revealed significant effects of timing and duration in all responses except female strobili in cuttings. Examination of residuals in Table 1 illustrates the more erratic response of cuttings compared to seedlings. The inconsistency of cuttings also obscured trends that were clearly evident in seedlings. Seedling responses indicate that the critical period for strobilus induction is protracted, and cannot be identified with any single 4-wk interval during April-July. In fact, best results were obtained for both male and female strobili when treatments were applied for 12 or 16 wk. Treatments commencing in April yielded consistently good results for male strobili. These conclusions were supported moderately well by the response of cuttings, although an 8-wk treatment (April-May) resulted in consistently high production of both male and female strobili. Control plants produced no strobili.

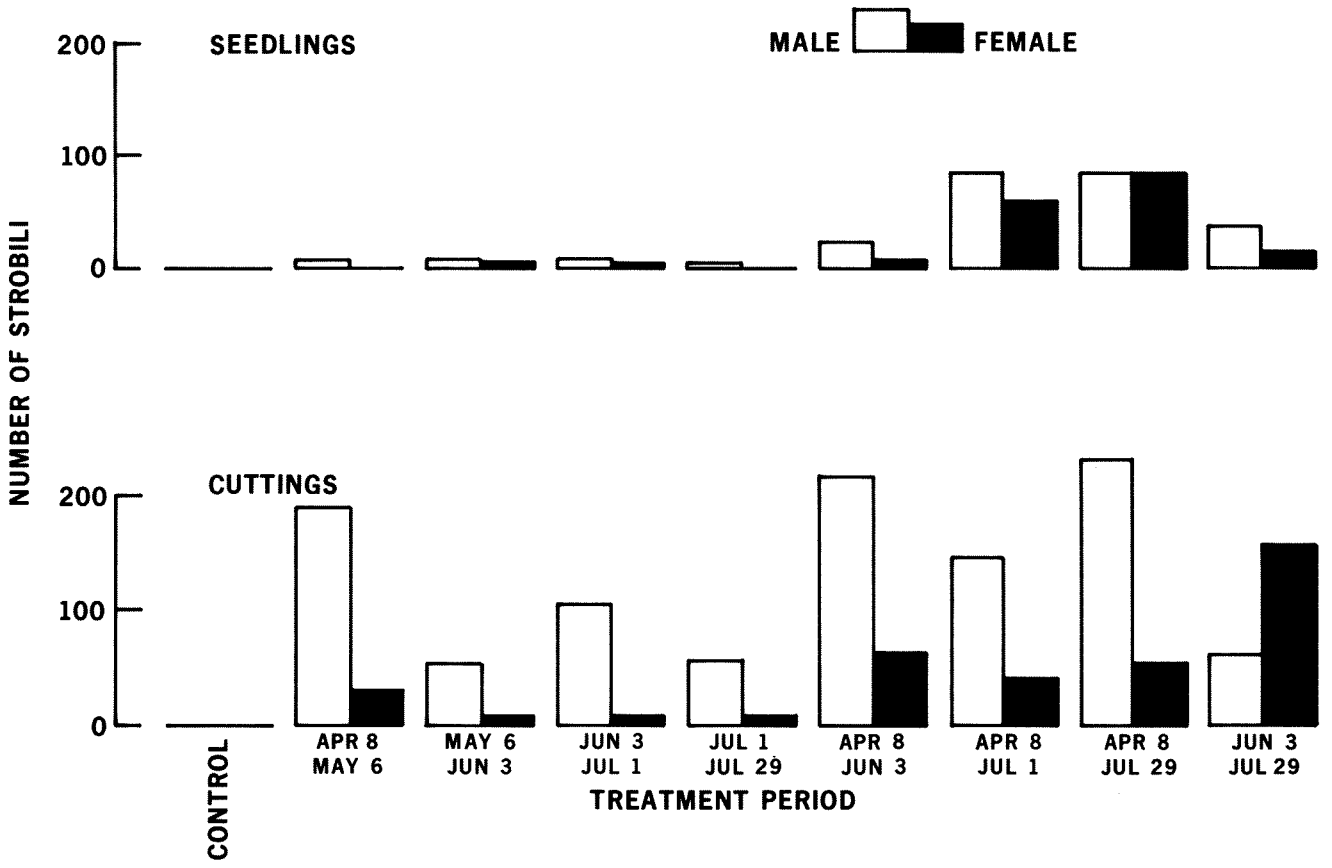

Figure 1. Effect of different periods of gibberellin and fertilizer treatment on average number of strobili produced in western hemlock plants.

TABLE 1

Analysis of variance of numbers of strobili produced on hemlock plants after different periods of treatment with gibberellin and fertilizer

Source	Degrees of freedom	Mean square	F-ratio
Male strobili on seedlings			
periods	7	10129	5.6*
blocks	4	2457	1.3
residual	60	1793	
Female strobili on seedlings			
periods	7	8417	3.6*
blocks	4	4010	1.7
residual	60	2344	
Male strobili on cuttings			
periods	7	21117	2.7**
blocks	4	12731	1.7
residual	60	7639	
Female strobili on cuttings			
periods	7	16969	1.1
blocks	4	21164	1.4
residual	60	14918	

* Significant at P=0.01
**Significant at P=0.05

The more advanced sexual maturity of rooted cuttings is reflected in their greater sensitivity to all treatments, with higher yields of strobili in most treatments. (Their greater sensitivity to toxic effects of treatments also resulted in 28 deaths, compared to a loss of 8 seedlings in the entire experiment.) On the other hand, variations in form and rooting pattern undoubtedly contributed to the erratic response of cuttings.

More male than female strobili were produced, differences being especially pronounced among cuttings subjected to short (4-wk) treatments, irrespective of the time of application. Male reproductive buds of this species complete differentiation naturally in the Victoria area in late June, compared to mid-July for female buds (Owens and Molder, Can. J. Bot. 52:283–294, 1974). The earlier differentiation of male strobili almost certainly explains their consistently high production following treatments commencing in April.

In conclusion, it would appear that while moderate crops of male strobili may be induced on rooted cuttings with gibberellin-based treatments as short as 4 wk,

reliable production of both sexes, in cuttings and seedlings, requires 12-wk treatments beginning in April. Although not tested, a shorter (8-wk) treatment beginning in early May might also be effective. The exact timing would depend in part on previous and prevailing temperatures because the greenhouse environment for this experiment undoubtedly hastened as well as heightened the receptivity of plants to treatments. Finally, there is some indication that timing of treatment can be used to manipulate the sex ratio of strobili induced by gibberellin because applications beginning in April invariably favored appearance of male strobili — D.F.W. Pollard, Canadian Forestry Service, Ottawa, Ont., and F.T. Portlock, Pacific Forest Research Centre, Victoria, B.C.

RECENT PUBLICATIONS JANUARY-MARCH 1983

5 **Alexander, Martin E. 1982.** Calculating and interpreting forest fire intensities. Can. J. Bot. 60:349–357.

7 **Auclair, Allan N.D. 1982.** Seasonal dynamics of nutrients in a *Carex* meadow. Can. J. Bot. 60:1671–1678.

6 **Bella, I.E., and J.P. De Franceschi. 1982.** Growth of lodgepole pine after mechanical strip thinning in Alberta: 15-year results. For. Chron. 58:131–135.

3 **Bonga, J.M. 1982.** Shoot formation in callus from the stalks of young female strobili of *Larix decidua*. Can. J. Bot. 60:1357–1359.

2 **Douglas, A.W., M. Alvo, and M.A.K. Khalil. 1981.** The use of multiple comparison tests in forest genetics research. Silvae Genetica 30: 193–196.

7 **Eis, S., D. Craigdallie, and C. Simmons. 1982.** Growth of lodgepole pine and white spruce in the central interior of British Columbia. Can. J. Forest Res. 12:567–575.

7 **Funk, A. 1982.** *Vanterpoolia*, a new genus of sporodochial hyphomycetes. Can. J. Bot. 60: 973–975.

6 **Joshi, S.R. 1982.** Airborne radioactive materials and plants: a review. Sci. Total Environ. 24: 101–117.

2 **Khalil, M.A.K. 1981.** Correlation of juvenile height growth with cone morphology and seed weight in white spruce. Silvae Genetica 30: 179–181.

6 **Lee, C.H. 1982.** Comparison of two correction methods for the bias due to the logarithmic transformation in the estimation of biomass. Can. J. Forest Res. 12:326–331.

3 **Loo, Judy, D.P. Fowler, and M.H. Schneider. 1982.** Geographic variation in specific gravity among Japanese larch from different provenances. Wood and Fiber 14:281–286.

3 **MacLean, D.A., and R.G. Lidstone. 1982.** Defoliation by spruce budworm: estimation by ocular and shoot-count methods and variability among branches, trees, and stands. Can. J. Forest Res. 12:582–594.

3 **Mahendrappa, M.K., and D.G.O. Kingston. 1982.** Prediction of throughfall quantities under different forest stands. Can. J. Forest Res. 12:474–481.

7 **Miller, Gordon E. 1982.** Strategies for control of insect pests in seed orchards. Pages 49–59 in Proc. Eighteenth Meet. Can. Tree Improv. Assoc. Duncan, B.C. 17–20 Aug., 1981.

7 **Morrison, D.J. 1982.** Variation among British isolates of *Armillaria mellea*. Trans. Br. Mycol. Soc. 78:459–464.

4 **Rey-Lescure, E. 1982.** Distribution des gourmands sur les fûts de 25 essences en bordure de déboisement. Can. J. Forest Res. 12:687–698.

7 **Reynolds, K.M., and W.J. Bloomberg. 1982.** Estimating probability of intertree root contact in second-growth Douglas-fir. Can. J. Forest Res. 12:493–498.

2 **Sidhu, S.S., and J.G. Zakrevsky. 1982.** A standardized method for determining buffering capacity of plant foliage. Plant and Soil 66: 173–179.

3 **Strunz, George M., and G. Sankar Lal. 1982.** Fittig bislactones in cyclopentenone synthesis: short synthesis of methylenomycin B. Can. J. Chem. 60:2528–2530.

3 **Strunz, George M., and Peter S. White. 1982.** On the synthesis of cryptosporiopsin: stereochemistry of early intermediates. Can. J. Chem. 60:2062–2064.

7 **Whitney, H.S., and O.J. Spanier. 1982.** An improved method for rearing axenic mountain pine beetles, *Dendroctonus ponderosae* (Coleoptera: Scolytidae). Can. Entomol. 114:1095–1100.

4 **Zarnovican, R. 1982.** Pertes en volume et caractéristiques dendrométriques du sapin baumier après défoliation par la tordeuse des bourgeons de l'épinette. Nat. can. 109:129–133.