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ENTOMOLOGY

Detecting Windthrow, Potential Foci for Bark Beetle Infestation, by Simple Aerial Photographic Techniques. — During endemic years, certain bark beetles, notably the spruce beetle, *Dendroctonus rufipennis* (Kirby), breed principally in wind-thrown trees, which are important in the development of periodic destructive outbreaks in British Columbia forests (Dyer and Taylor, Can. For. Serv. Inf. Rep. BC-X-62, 1971). Large areas of mass-wind-thrown trees, uprooted by violent windstorms, are easily seen and salvaged; but scattered wind-thrown trees, a few per hectare, are probably just as important as sources for bark beetle buildup. A method of detecting above-normal numbers of such trees that could be used by forest managers without expensive equipment or without personnel with special skill would be useful.

One method of quickly examining large areas in detail is that of using aerial photographs. Current experience is only with large, clearly evident patches of mass-wind-thrown trees (Moore, pages 338-346 *in* Proc. Second Can. Symp. Remote Sensing, Guelph, 1974; Moore, Can. Surv. 28:126-127, 1974; Murtha, Can. For. Serv. Publ. 1292, 1972). However, by comparing sequential aerial photographs of suitable quality and scale, single wind-thrown trees or gaps created in the canopy resulting from downed trees should be apparent.

To test the concept of using simple aerial photographic techniques to assess scattered windfall, a study was done in a mature white spruce–alpine fir–lodgepole pine stand east of Hixon, B.C., where an extensive spruce beetle outbreak occurred in the early 1960's.

Vertical, stereoscopic, black-and-white aerial photographs were taken through the open hatch of a de Havilland Beaver fixed-wing aircraft in June 1973 before and after the felling of 13 codominant trees to simulate windfall. The camera used was a hand-held, electric-drive 70 mm Hasselblad 500 EL/M, with 80 mm planar lens and 70-exposure magazine. A photo strip ranging up to 2.4 km in length was taken at each of three sample locations.

To study scale, photographs were taken at three altitudes: low, 150 to 145 m above ground level (AGL) (scale 1:2,000 to 1:3,000); medium, 450 to 1 000 m AGL (1:6,000 to 1:12,000); and high, 1 700 m to 1 900 m AGL (1:22,000). For easier comparison between the two sets of photographs (before and after felling), enlargements of up to 20 × 20 cm (8 × 8 in.) were used.

The two sets of photographs were compared to detect the felled trees. Searching was confined to 1 ha rectangular areas representing sample plots and outlined on the photographs around each group of felled trees. Search time was recorded for each examination of each set of imagery. The 11 technicians who examined the photographs were experienced aerial observers but not skilled photographic interpreters of forest damage, and were not specifically familiar with observations of windthrow.

The imagery taken at the lowest altitude proved unsatisfactory because of excessive distortion, too little frame overlap owing to inability of the camera to cycle frame fast enough, and poor control of aircraft flight path. This poor control, preventing duplication of the first photographs, was, in part, due to restricted visibility of the ground from the aircraft. Photography at the highest level, about 2 800 m AGL, was easiest to duplicate but proved too small a scale for seeing windthrow. At medium flying heights, flight-path control also was the main in-flight problem. Comparisons were possible, however, where the lines converged or where there was enough edge overlap.

The 11 technicians took an average of 18 min to search for the 13 felled trees on three stereo pairs of medium-scale photographs. On the average, they located 13 trees each, of which 10 were correct; the remaining three trees were old windfalls, or gaps shown in the crown canopy in the second set of photographs and identified in error as representing missing trees (Table 1).

TABLE 1

Observations of 11 technicians

Search time (min) of each observer	No. of felled trees detected	No. of other trees recorded
6	11	2
13	10	4
17	11	4
18	10	1
20	11	0
20	8	6
20	11	3
21	6	3
21	10	8
23	9	4
25	9	1
Average 18	10	3.2

Observers found that the difficulty of searching for missing trees on the two sets of photographs was due to variations in several factors:

1. *Scale*—Comparisons were simplified if scale was nearly the same for each set of photographs; the photographs could then be viewed together stereoscopically. This rarely occurred directly, because flying height was never the same, but the scale could be partly adjusted by enlarging one or the other photograph.
2. *Resolution*—Enlargements were subject to resolution problems, particularly when low available light required a slow shutter speed, with resultant image motion.
3. *Angle of view*—Areas of interest on the imagery almost always were photographed from slightly different angles in successive instances. Comparisons between successive photographs were consequently difficult.

The method, while showing promise, needs improvement to become operational. Using a helicopter, rather than a fixed-wing aircraft, would reduce most of the problems. The less expensive fixed-wing aircraft requires more sophisticated control of location and scale than seems possible with a hand-held, small-camera system; most forest managers would probably employ a contractor specializing in this field. An alternative to aircraft, in suitable terrain, would be fixed, ground-based camera stations with a clear view of the stands. Color film, rather than black-and-white, might be worthwhile in spite of greater processing difficulties, additional expense, and narrower exposure latitude, as it would assist relatively unskilled interpreters in seeing greater detail. — J.W.E. Harris, A.F. Dawson, and R.G. Brown, Pacific Forest Research Centre, Victoria, B.C.

Sampling Overwintering Spruce Budworm Populations in Heavily Attacked Stands. — In cage experiments, spruce budworm females, *Choristoneura fumiferana* (Clem.), prefer to oviposit on nondefoliated shoots (Miller, page 75 *in* Mem. Entomol. Soc. Can., 1963). This led to the supposition that in heavily attacked stands more eggs might be laid on trees with the least defoliation (where defoliation refers to the loss of both current and old needles). Although this has never been checked, we have counted L2 larvae on trees in the same stand with differing degrees of defoliation.

The Maritimes Forest Insect and Disease Survey collected three midcrown branches from each of 20 trees in a balsam fir, *Abies balsamea* (L.) Mill., stand on Cape Breton Island. The collection area covered about 1.0 ha. The stand had been under heavy budworm attack and total defoliation ranged from 20 to more than 90% of the needles, and some trees were dead. Foliage samples were collected from 10 trees with 50% or

TABLE 1

Counts of overwintering spruce budworm larvae on trees with moderate total defoliation and trees with severe total defoliation, Cape Breton Island, 1977-78 generation

Tree	m^2 of foliage	Total defoliation (%)	Number of budworm	
			Total*	Per m^2
1	0.87	90+	16	18
2	0.85	90+	24	28
3	0.56	90+	17	30
4	1.12	90+	36	32
5	1.33	90+	21	16
6	0.88	90+	26	30
7	0.99	90+	18	18
8	0.62	90+	14	23
9	1.17	90+	33	28
10	0.51	90+	39	77
Mean			24	300
11	0.98	40	64	65
12	0.76	40	49	65
13	0.77	30	57	74
14	0.84	50	37	44
15	0.87	40	84	97
16	0.87	30	38	44
17	1.11	30	67	60
18	1.25	30	103	82
19	0.72	20	87	121
20	1.14	30	66	58
Mean			65	710

*Three midcrown branches.

less defoliation and 10 trees with 90% or more defoliation. The samples were washed in NaOH to remove the L2 larvae (Miller and McDougall, Bi-mon. Res. Notes 24:30-31, 1968).

Except for tree 10, the mean number of larvae per m^2 of foliage ranged from 16 to 32 on the severely defoliated trees and from 44 to 121 on the moderately defoliated trees (Table 1). The significant difference in the mean L2 counts suggests that samples should be stratified into at least two defoliation classes when L2 abundance in severely attacked stands is being measured. The data also imply that a similar type of stratification should be investigated in sampling for budworm eggs. — C.A. Miller, Maritimes Forest Research Centre, Fredericton, N.B.

Fractionation of Spruce Budworm Testes Cells by Velocity Sedimentation.—Determination of the stadial sensitivity of germ line cells to mutagens and chemosterilants requires a rapid and efficient method of separating cells on the basis of their stage of development. This is particularly so for the spruce budworm, whose spermiogenesis becomes asynchronous in the fifth instar (Retnakaran, Ann. Entomol. Soc. Am. 63:851-859, 1970); from that point on, testes contain variable proportions of stages from spermatogonia to sperm. With such asynchronous development, the usual techniques of microdissection or centrifugation produce cell populations of only partial purity and/or low yield. Our studies on the effects of chemical treatments on chromosome structure and sperm maturation have necessitated development of a rapid method for isolating the desired cell stages at a high level of purity.

The procedure takes advantage of the fact that at unit gravity cells sediment through a nonlinear low-density gradient at a rate dependent primarily on their size, though density plays a minor role (Miller and Phillips, J. Cell. Physiol. 73:191-201, 1969). The gradient is present only to prevent convection; at no point is it sufficiently dense for any cells to approach their equilibrium density. The method was adapted from that used for mouse testes cells (Lam et al., Proc. Natl. Acad. Sci. U S A 65:192-199, 1970), with modification to accommodate the smaller amounts of tissue available and the particular physiological requirements of lepidopteran cells.

The apparatus consists of a cylindrical separation chamber with a conical bottom having a hole at the apex of the cone, a sample-loading chamber, and a gradient-former. Luer-tip equipped glass syringes of 5 to 100 mL capacity make excellent separation chambers. A 1 to 5 mL syringe equipped with a two-way valve serves as a cell-loading chamber. A Beckman gradient-former of 5 to 100 mL capacity is connected through the two-way valve to the separation chamber; the valve permits loading of the sample into the separation chamber and then formation of the gradient. Connecting tubing is autoclavable silicone.

Description of a representative separation run will illustrate the procedure. Pooled testes from five fifth-instar spruce budworm larvae were gently minced with fine iris scissors in 10 volumes of modified Castillo-Ringer (C-R) (Castillo et al., J. Physiol. 121:539-547, 1953) until a uniform cell suspension was obtained. Cells were rinsed once in C-R and then resuspended in 1% Ficoll 400 (Pharmacia) in C-R. Cell concentration was adjusted to *ca* 10^6 cells/mL, which gave a final volume of 0.9 mL for the cell suspension. Sufficient 0.5% Ficoll in C-R was run into a 20 mL glass syringe to fill the cone and break the meniscus. The cell sample was then loaded from the bottom at a rate of 0.5 mL/min. This was followed by 20 mL of a 2-3% Ficoll gradient. The gradient lifted the cells to form a thin band near the top of the chamber. When loading was complete, the cells were allowed to sediment at 4° C in a vibration-free cold room. After 4 h the cone volume was discarded and 1 mL fractions were collected from the bottom. Cell types in each fraction were determined from slides prepared directly from the fraction with a Shandon Cyto-Centrifuge fixed in 3:1 methanol-acetic acid and stained with 2% acetocarmine in 45% acetic acid.

TABLE 1

Sedimentation separation of germ cells of male spruce budworm

Fraction[1]	Cell type[2]
1	Large, indeterminate identity
2	Pigmented epithelial
3-5	Few, indeterminate identity
6-7	Pachytene; diplotene
8-9	Diplotene; MI; early meiotic prophase
10	Meiotic prophase of undetermined stages
11-13	Spermatogonia; apparently somatic cells of indeterminate origin
14-15	Secondary spermatocytes; MII; early spermatids; spermatogonia
16	Spermatids; late secondary spermatocytes
17	Spermatids
18	Sperm; spermatids
19	Sperm; very few spermatids
20	Cellular debris

[1] Twenty fractions of 1 mL collected from bottom of separation chamber.
[2] All fractions contained minor components of cells of indeterminate identity.

Sedimentation rates of cells are related to their volume (Lam et al., 1970) and resolution is therefore greatest in the upper- and lowermost fractions (Table 1). The large primary spermatocytes sediment rapidly, while the small spermatids and sperm sediment slowly if at all. The best resolution between adjacent fractions is obtained with sperm and spermatids; the respective fractions are about 95% free of other contaminating cell types. There is some overlap in cell type among fractions in the middle of the gradient, due in large part to the similarity in size of spermatogonia, secondary spermatocytes, and an indeterminate cell type that may originate in the testes tunica. However, some fractions are clearly enriched in identifiable cell types, e.g. fractions 11 to 13 in spermatogonia and fractions 14 and 15 in secondary spermatocytes.

The relative positions of cell types in the gradient are consistent from run to run, though absolute position may differ by a fraction or two. Proportions of cells of each type are, of course, a function of age of donor insects, spermatogonia and spermatocytes predominating in younger larvae and spermatids and sperm predominating in pupae (Retnakaran, 1970). With the appropriate choice of medium, cell viability and morphology remain unaffected by runs of up to 6 h duration. Spruce budworm spermatogonia isolated by fractionation continued to incorporate tritiated thymidine into their DNA when postincubated in a labelled medium.

The technique has been successfully applied to as little tissue as a single pair of sixth-instar spruce budworm testes, to a number of other insect species, and to cells from established lepidopteran cell lines. With appropriate scaling to the number of cells available, it would appear to be applicable to any tissue from which a cell suspension can be obtained.— T.J. Ennis and N. Charlebois, Forest Pest Management Institute, Sault Ste. Marie, Ont.

Effect of Seudenol on Spruce Beetle and Douglas-fir Beetle Aggregation.—The effect of seudenol, a pheromone of the Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopk.) (Vité et al., Naturwissenschaf-