Tree Ring Evidence for Chronic Insect Suppression of Productivity in Subalpine *Eucalyptus*

Abstract. *Experimental reduction of insect grazing pressures suggests that long-term, continuous feeding by phytophagous insects has severely depressed growth and productivity of subalpine* Eucalyptus *trees (measured by annual ring growth) for at least 25 years.*

Eucalyptus species in their native Australian habitats are subject to heavy, more or less continual attack (*1*) by numerous species of insects (*2*). We now report that there is a dramatic increase in growth rate in two species of *Eucalyptus* following insecticide treatment for chronic insect infestation. The width of annual rings indicates that insect attack has depressed growth rates in *Eucalyptus* over long time spans (Fig. 1). Although some studies have shown that major insect outbreaks decrease radial growth in eucalypts and other species of trees (*3, 4*), the effects of continual attack has never been shown.

Our study was carried out in an even-aged subalpine woodland (*5*) in the Snowy Mountains of southeastern Australia. Three trees each of *E. pauciflora* and *E. stellulata* were selected for insecticide treatment in the summer of 1972 (the ring for 1972 represents the 1972–1973 growing season). Most were small and had two or more stems that branched off near the ground surface. Between December 1972 and March 1973, the foliage on one stem per tree was sprayed weekly (*6*) with Thiodane (a broad spectrum, nonpersistent, nonsystematic, contact insecticide) while a temporary shield protected the foliage of the rest of the tree from receiving insecticide. During the next 3 years the condition of the trees was monitored, but no further insecticide treatment was given. In April 1976, the sprayed stems of each of the six treated trees were cut, and a stem of comparable size and exposure was cut from the unsprayed part, to provide slices for dendrochronological studies. In July 1976, slices were also taken from two stems of each of 11 control trees from the same site, which had not been included in the spraying program.

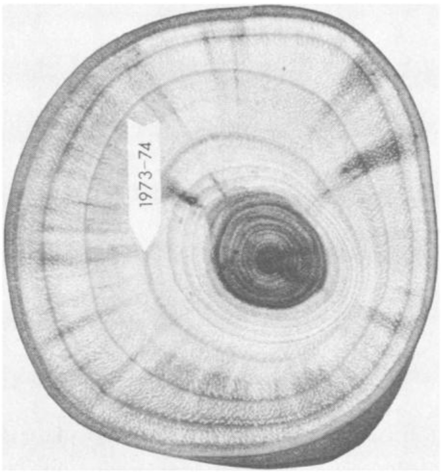

Fig. 1. The effect of reduced insect attack is seen in the years following treatment in this cross section of *Eucalyptus stellulata*. A large increase in width of annual growth rings followed insecticide treatment of foliage in the 1972–1973 growing season. Arrow shows ring boundary between the 1972–1973 and 1973–1974 growing seasons; the outermost ring is that for 1975–1976; the dark rim is bark; the branch is 18 years old.

We tested the hypothesis that freedom from insect attack would influence productivity (as measured by radial growth) by comparing the average growth of *E. stellulata* and *E. pauciflora* prior to spray treatment with the rates for the period after the spraying. Growth rates for the same subperiods in the untreated control trees were similarly compared to test whether any apparent changes could be due to more general fluctuations in environmental conditions unrelated to the treatment. The annual-ring sequence on one radius of each slice was measured as far back as it was possible to date accurately (*7*). All specimens contain a distinctive frost zone (*8*) in the 1967 annual ring, and therefore we used this ring as the starting point for a comparison of increment growth before and after treatment. For each slice, ring widths were converted to dimensionless growth indices (*9*), and a mean ring-width index was computed for two subperiods of each radial series. The subperiod preceding treatment was considered to be that from 1967 through 1972; that after treatment was considered to be from 1973 through 1975. The subdivision for the sequence after treatment (1973 to 1975) is based on the pronounced increase in ring width in treated trees beginning with the annual ring for 1973. Although insecticide was used only in the 1972 season, there was little apparent effect on the width of the ring formed in that season.

For each *Eucalyptus* species we compared, for three groups, indices after treatment to those before treatment: (i) branches of control trees, (ii) sprayed branches of treated trees, and (iii) unsprayed branches of treated trees (Fig. 2). There were large increases in growth rates of sprayed branches of *E. stellulata* [$P < .001$, based on a two-tailed t-test (*10*) against a null hypothesis of no change in the mean value of the ring width] and of *E. pauciflora* ($P < .001$). There was also a parallel, but more modest, increase in unsprayed branches of treated trees on both *E. stellulata* ($P < .001$) and *E. pauciflora* ($P < .05$). In contrast, data for the control group show that there was no change in growth rates of untreated *E. stellulata* between 1967 and 1972 and between 1973 and 1975. Untreated *E. pauciflora*, however, showed a significant ($P < .05$) decrease in growth rates between these two time periods (*11*).

The average annual growth increments in control trees for 1950 through 1975 and for treated trees from 1967 through 1975 are shown in Fig. 3. The similar growth indices in control and treated trees prior to 1973 contrasts with the difference in indices after that year and indicates that insect attack has suppressed productivity over long periods of time.

Ring widths were slightly larger in sprayed trees than in control trees in the season of treatment (1972), but the maximum response is evident 1 or 2 years later (Fig. 3). The increases in ring width in treated trees is consistent with the increase in foliage that resulted from insecticide treatment (*12*). During the season that trees were sprayed, only the treated branch showed a luxurious increase in foliage. During the following season, however, the entire tree showed much enhanced canopy development. This response of the entire treated tree

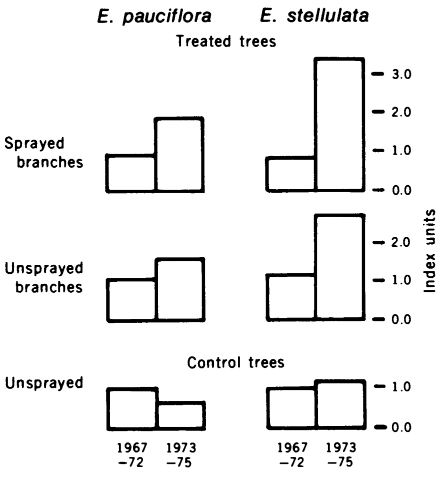

Fig. 2. Mean ring-width indices for periods before (1967 to 1972) and after (1973 to 1975) treatment are plotted for insecticide-treated *E. pauciflora* ($N = 5$) and *E. stellulata* ($N = 3$) and unsprayed control trees of *E. pauciflora* ($N = 6$) and *E. stellulata* ($N = 5$).

during the second season is mirrored in the annual ring growth (Figs. 1 and 3); unsprayed branches had somewhat smaller growth increments than sprayed branches in the second and later seasons, but both were far greater as compared to the controls (*13*). Increased annual ring growth in sprayed trees persisted from the year following treatment until the trees were cut at the end of the 1975 growing season. The effect, however, decreased after the first year for *E. stellulata* and after the second year for *E. pauciflora*: the downward trends in ring width (Fig. 3) suggests that by 1976 or 1977 increment growth in both species would have returned to the average value seen in control trees.

The growth rates of both eucalypt species were significantly increased by removal of phytophagous insects (Fig. 2). Three observations suggest that *E. stellulata* is much more heavily attacked by insects than was *E. pauciflora*: (i) the increase in ring width after the spray treatment was nearly two times greater in *E. stellulata* than in *E. pauciflora*, (ii) that growth rates began to decrease sooner in *E. stellulata* (Fig. 3) suggests a more rapid buildup of insect numbers after insecticide treatment stopped, (iii) there was a much greater response of foliage to spray treatment in *E. stellulata*. These observations are consistent with other studies showing that *E. stellulata* loses more leaf tissue when unprotected by insecticides and has a larger number of host specific insect species and higher total population densities of insects than *E. pauciflora* (*14, 15*). However, while the above information indicates a greater positive response to herbivore removal in *E. stellulata*, the data (Figs. 2 and 3) may in part be explained by differences in the total volume of foliage sprayed since this factor could not be closely controlled.

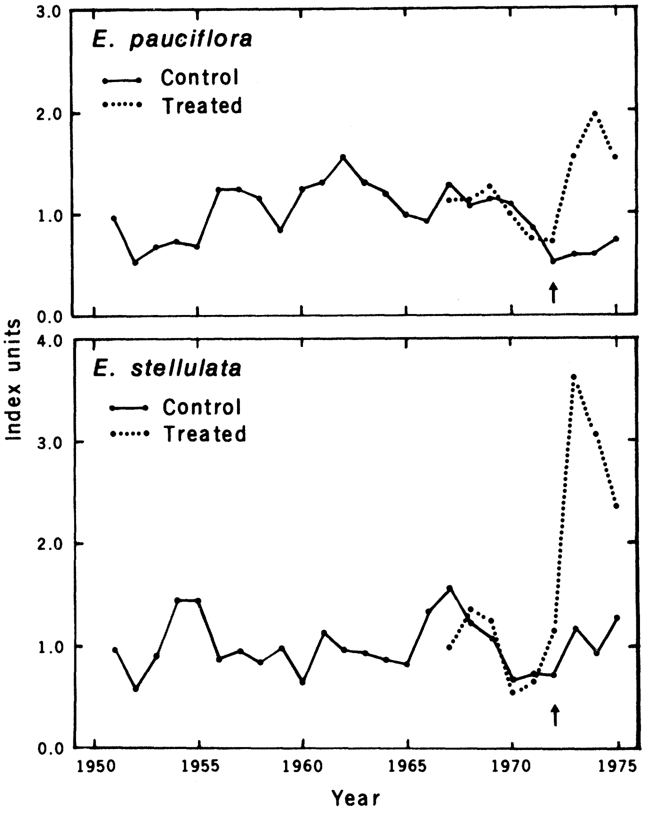

Fig. 3. Long-term growth records in subalpine eucalypts indicate chronic suppression of productivity by phytophagous insects. Annual mean ring-width indices, 1951 to 1975, in 11 control trees are compared with indices in 8 treated specimens, 1967 to 1975. Growth trends in treated specimens prior to insecticide spraying (arrow) are similar to those in untreated controls. Data for sprayed and unsprayed branches of treated trees are combined.

Our major conclusion is that the productivities of these *Eucalyptus* species are strongly suppressed by phytophagous insects and that this suppression occurs over long periods of time. At no point during the last 25 years did growth rates approach those levels attained after partial removal of phytophagous insects. Loss of large amounts of tissue appears to be a constant factor among these eucalypts. In this subalpine habitat as well as in other areas of Australia, average tissue loss to insects has been estimated at between 20 and 50 percent, and complete defoliation was not uncommon (*1, 4, 16–18*). These levels of attack, when combined with the high degree of host selectivity demonstrated by some herbivores feeding on eucalypts (*14–16*), may differentially affect competitive abilities of these trees and may influence their distributions (*19*).

Research designed to factor out biological interference from growth records have been developed for the rare, episodic event (*3*), and it is not clear that they can be used where insect effects on productivity are continual. This major difficulty combined with the problems encountered in dating eucalypt growth rings (*8*) suggest that it may prove difficult to reconstruct past climate from *Eucalyptus* growth-ring sequences. This is unfortunate because *Eucalyptus* species initially appeared to be ideal material for paleoclimatological studies. Some eucalypts are very large and apparently old, and occur in marginal habitats where good paleoclimatic sensitivity would be expected. *Eucalyptus*, with some 600 to 700 species (*20*), so dominates mainland Australian forests and woodlands that alternative dendrochronological material is difficult to find. However, the demonstrable impact of long-term phytophagous insect attack on ring-width growth shown in our study and in studies of insect outbreaks (*4, 17*) means that natural fluctuations in insect populations could account for much of the variability seen in long time series of ring widths from trees of this genus and could be more important than the direct limiting effects of climate. However, to the extent that insect population levels are determined by weather conditions, an indirect link between climate and tree growth might be established.

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References and Notes

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3. T. W. Koerber and B. E. Wickman, *Univ. B.C. Fac. For. Bull.* **7**, 101 (1970).
4. J. L. Readshaw and Z. Mazanec, *Aust. For.* **33**, 29 (1969); Z. Mazanec, *ibid.* **32**, 3 (1968).
5. The study site is near Dainer's Gap in Kosciusko National Park, New South Wales (36°12'S, 148°43'E; 1585 m, altitude). It is an open subalpine woodland dominated by *E. pauciflora* (snow gum), *E. stellulata*, and *E. perriniana*, with an understory of shrubs and a ground cover of grasses and sedges. The site slopes moderately (20° to 25°) northward, and the deep soil is developed from granitic parent material. The area was burned in 1939, and above-ground portions of most trees were killed. Age estimates based on ring counts show that most of the trees in the area today originated as shoots sprouting from burned stumps (coppice) or became established from seedlings that germinated in the decade after the fire. Typical trees are low (3 to 10 m), and those reestablished from coppice have multiple branching stems. The area has been protected from grazing by domestic animals at least since the fire, and at this altitude there are no marsupials which eat *Eucalyptus* [A. B. Costin, *Ecosystems of the Monaro Region of New South Wales* (Government Printer, Sydney, 1954); D. Wimbush, personal communication].

6. Trees were sprayed weekly in spring when shoot expansion was greatest. After mid-January, trees were sprayed fortnightly. Concentrations of insecticide and spreader were those suggested by the manufacturer.
7. All the samples could not be dated with the same degree of confidence prior to 1967 (8). However, from most of the samples we were able to obtain accurate long-term datings.
8. Most eucalypts are difficult or impossible to date because of poorly defined annual ring boundaries, a high frequency of intra-annual latewood bands (false rings), and a high percentage of parenchyma tissue that obscures or completely masks the discontinuities in tracheid characteristics defining successive growth rings. In slow growing eucalypts and those subject to severe defoliation from drought, fire, or insect attack, some growth rings may be extremely narrow or even locally absent from parts of the tree's circumference (4, 17). However, because of the distinct seasonality of the subalpine environment, *E. stellulata* and *E. pauciflora* are comparatively easy to date by means of standard dendrochronological techniques [M. A. Stokes and T. L. Smiley, *An Introduction to Tree-Ring Dating* (Univ. of Chicago Press, Chicago, 1968)] of counting and cross-dating. In addition to cross-matching of characteristic narrow rings representing the synchronous response of tree growth to shared environmental conditions in certain years, chronological accuracy was ensured by recognition of a distinctive pattern of frost-damage zones that result from freezing of the stem during or shortly after the period of cambial activity [V. C. LaMarche, Jr., *Univ. B.C. Fac. For. Bull.* 7, 99 (1970)]. Because a frost-damage zone records a short spell of unseasonably cold weather during the growing season, it provides a very valuable internal dating check. Within our study area, a distinctive double frost zone is usually contained in the 1967 ring.
9. Ring widths are normally converted to dimensionless growth indices. To obtain these indices we measured ring widths along the radius of maximum length between pith and bark on each slice and divided each value by the mean ring-width value for the period between 1967 and 1972 for that radius.
10. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969).
11. A period of declining growth began about 1970 in *E. pauciflora* (Fig. 3).
12. Eucalypts are evergreen, and in these species leaf life is 13 to 15 months. An increase in foliage will therefore make a longer term contribution to plant productivity than if the leaves were shed each autumn.
13. The small response in growth increment in the season of treatment suggests that either annual ring growth draws heavily on stored photosynthate or that annual ring formation had been essentially completed by the time the foliage crop resulting from the spray treatment began to contribute exportable quantities of photosynthate. Phenological observations indicate that the growing season begins in November [R. O. Slatyer, *Oecologia* **24**, 357 (1976)] and lasts through March. By late December of the year of treatment (1972), a large increase in leaf area had occurred in response to the spray treatment and increment cores taken at this time showed that annual ring growth was incomplete. Since cambial activity is not restricted to the early part of the growing season it is difficult to explain the virtual lack of ring-width response to increased foliage area immediately following treatment. A possible explanation is that current photosynthate is used for local canopy building and is neither translocated to the canopy of other stem systems [M. G. Cook and L. T. Evans, in *Transport and Transfer Processes in Plants*, I. F. Wardlaw and J. B. Passioura, Eds. (Academic Press, New York, 1976), p. 393] nor used for ring formation during that growing season. Rather, ring growth may utilize photosynthate stored after canopy construction is completed for the season [H. C. Fritts, *Tree Rings and Climate* (Academic Press, London, 1976)]. This explanation assumes that much of the photosynthate is stored in the roots over winter and is redistributed more or less equally among all stems during the following year, thus increasing foliage volumes and increment growth throughout the tree.
14. These eucalypt species are attacked primarily by sawflies (Hymenoptera: Pergidae) and numerous species of Hemiptera and Coleoptera. Of 48 species commonly attacking *E. stellulata*, more than one-half ate only this food plant. On *E. pauciflora*, 39 species were common and 28 percent of these were host-specific. The total number of individuals in the samples from *E. stellulata* was 60 percent greater than from *E. pauciflora*. Details of collecting methods and species identifications are given in (15). Measurements of insect damage were made in 1975–1976. *Eucalyptus stellulata* lost 96 percent of its shoots and 50.5 percent of its leaf area to chewing insects, a significantly greater loss than the 76.8 percent of shoots and 36.7 percent of leaf area in *E. pauciflora*. This measure of damage did not include loss of photosynthate to sucking insects, which were much more common on *E. stellulata* (15).
15. P. A. Morrow, *Aust. J. Ecol.* **2**, 89 (1977).
16. J. J. Burdon and G. A. Chilvers, *Aust. J. Bot.* **22**, 103 (1974).
17. Z. Mazanec, *Aust. For.* **37**, 32 (1974); L. R. Clark and M. J. Dallwitz, *Aust. J. Zool.* **23**, 523 (1975).
18. P. B. Crane, *Aust. J. Zool.* **14**, 647 (1966); R. G. Greaves, *Appita* **19**, 119 (1966), G. A. Kile, *Aust. For. Res.* **6**, 9 (1974); M. M. H. Wallace, *Aust. J. Zool.* **18**, 91 (1970); J. L. Readshaw and Z. Mazanec, *Aus. For.* **33**, 29 (1969). These levels of tissue loss are much greater than any estimate for temperate Northern Hemisphere trees where they range from 3 to 10 percent leaf area loss per year with occasional outbreaks of insects causing complete defoliation [R. J. Bray, *Ecology* **45**, 165 (1964); C. W. Elton *The Pattern of Animal Communities* (Methuen, London, 1966); J. R. Gosz, G. E. Likens, F. H. Bormann, *Ecology* **53**, 769 (1972); W. J. Mattson and N. D. Addy, *Science* **190**, 515 (1975)]. While repeated destruction of large amounts of foliage may eventually lead to high mortality (17), most *Eucalyptus* spp. are very resistant to defoliation and have features that imply a long evolutionary history of defoliation by fire, drought, herbivores, or all of these factors [M. R. Jacobs, *Growth Habits of the Eucalypts* (Commonwealth of Australia Forestry and Timber Bureau, Canberra, 1955)].
19. P. A. Morrow, in preparation.
20. L. D. Pryor and L. A. S. Johnson, *A Classification of the Eucalypts* (Australian National University, Canberra, 1971).
21. We thank the Centre for Resources and Environmental Studies, the Department of Biogeography and Geomorphology and the Department of Environmental Biology, Australian National University, for laboratory space and logistic support, G. Robinson and D. A. Campbell for technical assistance and L. R. Fox, H. Fritz, D. C. Potts, and G. D. Tilman for comments on the manuscript. Supported by the Climate Dynamics Program, Division of Atmospheric Physics, U.S. National Science Foundation. Permission to work in Kosciusko National Park was granted by the New South Wales National Parks and Wildlife Service.

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Brain Tumors in Owl Monkeys Inoculated With a Human Polyomavirus (JC Virus)

Abstract. *Owl monkeys were inoculated intracerebrally, subcutaneously, and intravenously with JC, BK, or SV40 virus. Two of four adult owl monkeys inoculated with JC virus, a human polyomavirus, developed brain tumors at 16 and 25 months after inoculation, respectively. A grade 3 to grade 4 astrocytoma (resembling a human glioblastoma multiforme) was found in the left cerebral hemisphere and brainstem of one monkey. The second monkey developed a malignant tumor in the left cerebral hemisphere containing both glial and neuronal cell types. Impression smears prepared from unfixed tissue of this tumor showed cells that contained polyomavirus T antigen. Virion antigens were not detected. Tumor cells cultured in vitro also contained T antigen but were negative for virion antigen. Infectious virus was not isolated from extracts of this tumor.*

Man is the natural host for two polyomaviruses: JC virus (JCV) and BK virus (BKV). Cryptic infection with JCV and BKV usually occurs during childhood and is a common event (1). The JCV has been isolated from brain tissues of patients with progressive multifocal leukoencephalopathy (PML) and is the virus usually associated with this demyelinating disease (2). The BKV has been isolated from urine of immunosuppressed patients (3). However, this virus has not been associated with any known disease. Infection in man by the simian polyomavirus SV40 has been reported. A major exposure occurred some years ago when a large number of people were inadvertently inoculated with poliovirus vaccine contaminated with SV40. A variant of this virus, SV40-PML was isolated in two cases of PML (4). Tumors have been induced in Syrian hamsters by JCV, BKV, SV40, and SV40-PML. The JCV is particularly notable for its strong predilection for producing tumors in the nervous system. Tumors induced by JCV in the hamster include medulloblastoma, undifferentiated neuroectodermal tumor, glioblastoma, ependymoma, pineocytoma, neuroblastoma, and meningioma (5). In contrast, BKV and SV40-PML induce only tumors of ventricular surfaces identified as choroid plexus papillomas or ependymomas (6, 7).

Because of the exposure of large numbers of people to these viruses, and because of the association of these viruses with PML and their ability to induce tumors in hamsters, we decided to test their oncogenicity in subhuman primates. Adult owl monkeys were chosen for the first experiment because they had low or undetectable antibodies against JCV, BKV, and SV40. Serum from other New World monkeys had detectable antibody against SV40 and occasionally JCV. Adult feral Colombian owl monkeys (*Aotus trivirgatus*) were inoculated in the following manner. A small hole was made in the skull over each cerebral hemisphere by means of a dental drill (0.75-mm stainless steel bit). The inoculum, 0.15 ml per hemisphere, was de-