

Leaf nitrogen and its effects on the rate of herbivory on selected eucalypts in the jarrah forest

G. Casotti^a and J.S. Bradley^b

^aSchool of Veterinary Studies, Murdoch University, Murdoch, W.A., 6150, Australia

^bSchool of Biological Sciences, Murdoch University, Murdoch, W.A., 6150, Australia

(Accepted 12 January 1990)

ABSTRACT

Casotti, G. and Bradley, J.S., 1991. Leaf nitrogen and its effects on the rate of herbivory on selected eucalypts in the jarrah forest. *For. Ecol. Manage.*, 41: 167–177.

This study measured the relationship between the rate of leaf consumption by insect herbivores and the level of leaf nitrogen in selected species of eucalypts. Sampling was carried out on a monthly basis from *Eucalyptus accedens*, *Eucalyptus resinifera* and *Eucalyptus marginata* in areas rehabilitated following bauxite mining and from *E. marginata* in a forest regenerated after clearfelling. The rate of herbivory was examined on leaves aged 1–3 years. The mean monthly rate of herbivory of all species varied from 0.9 to 1.9% of the total leaf area and was positively correlated ($P < 0.05$) with the level of leaf nitrogen in *E. marginata*. The previous month's level of nitrogen was also positively correlated to the rate of herbivory in the current month in all species. The level of leaf nitrogen was negatively correlated to the current and previous month's level of herbivore abundance and the current month's rate of herbivory in *E. resinifera*. These correlations suggest two possibilities. Firstly, that in some eucalypts the level of leaf nitrogen may be lowered when the level of predation on leaves is high. Secondly, that herbivorous insects may preferentially feed on eucalypt leaves having a high amount of nitrogen.

INTRODUCTION

A number of studies have suggested that the rate of foliage consumption by insect herbivores in Australian eucalypt forests is greater than in temperate forests in the northern hemisphere (Kile, 1974; Carne et al., 1974; Mattson and Addy, 1975; Morrow, 1977; Springett, 1978; Journet, 1981). Reported annual foliage losses in some Australian eucalypts range from 5 to 70% (Journet, 1981; Lowman, 1982; Fox and Morrow, 1983), compared with a maximal level of 15% in northern hemisphere *Quercus* forests (Bray, 1961, 1964) and in a lowland tropical rainforest (Coley, 1983). Foliage removal by herbivorous insects may alter the growth rates or competitive success among

plants (Fox and Morrow, 1986) by removing leaf area which could otherwise be used to generate energy by photosynthesis.

Changes in the nutrient content of leaves have been associated with affecting both the survivorship and the amount of defoliation by insect herbivores (Morrow, 1983). Nutritional value varies with the amount of nitrogen, moisture and fibre as well as the concentration of allelochemic compounds within the leaf (Mattson, 1980). Nitrogen has been shown to affect the growth rate of lepidopteran larvae (Rodríguez, 1960; Feeny, 1970; Slansky and Feeny, 1977) and the growth rate (Onuf et al., 1977) and feeding efficiencies of coleopteran larvae (Fox and Macauley, 1977). Leaf defoliation has also been shown to be associated with increases in the level of total protein nitrogen (Wareing, 1970; McNaughton, 1979), however, Fox and Macauley (1977) found no evidence of this in Australian eucalypts. Tuomi et al. (1984) reported that insect defoliation reduced the leaf nitrogen content in mountain birch, while increasing the levels of phenolic compounds. In contrast, Coley (1983) found no evidence of an increase in the level of phenolic compounds with leaf predation.

Nitrogen, which is a source of protein for insects, occurs in low concentrations in plant leaves (Slansky and Feeny, 1977). Soo Hoo and Fraenkel (1966a,b) suggested that plants with less than 3% nitrogen are substandard food for herbivores. By this criterion, the vast majority of native Australian eucalypts are substandard. Therefore, to obtain adequate amounts of nitrogen in their diet herbivorous insects must consume relatively large quantities of tissue (Crawley, 1983). The removal of a large amount of leaf material may cause stress within the plant. Stress arising from both insect herbivore predation and variations in climatic conditions has been associated with an increase in the level of leaf toughness and fibre content in lowland tropical rainforest plant species (Coley, 1983) and an increase in the available leaf nitrogen (White, 1984). However, stress caused by leaf predation by insect herbivores might have the effect of lowering the level of leaf nitrogen. The purpose of this study was to examine the relationship between leaf nitrogen, herbivore abundance and herbivory rate on different species of eucalypts.

METHODS AND MATERIALS

The study area

This study was undertaken in the south west of Western Australia. Two sites, one containing *Eucalyptus accedens* and *Eucalyptus resinifera* and the other *Eucalyptus marginata*, were located in areas rehabilitated by tree planting following bauxite mining. A third site located in a eucalypt forest, consisted predominantly of *E. marginata*.

The sample size consisted of three trees of *E. accedens* and *E. resinifera* and

in the case of *E. marginata*, three trees from each of the two sites in which it occurred. Sampled trees were 2–4 m in height.

Herbivore collection

Insect herbivores were sampled once each month by both tree beating and net sweeping. Insects were classified as herbivores if any stage of their life history was known to affect the survival of plant foliage by physical removal (i.e. chewing and rasping) or sucking tree sap.

The rate of herbivory

The rate of herbivory was defined as any area lost within, or on the outer perimeter of the leaf over a monthly period. Ten healthy leaves, commencing with the youngest fully emerged leaf from the previous year, were tagged on three branches of each tree. The rate of herbivory on each leaf was monitored over an 8 month period, by tracing the outline of any area missing within the leaf onto transparent plastic sheets. A planimeter was then used to measure the area of each leaf and a loss in leaf area between samples was assumed to be caused by herbivory.

The amount of leaf area lost was not adjusted for leaf expansion because the growth of these leaves was found to be minimal using the statistical model derived by Reichle et al. (1973).

Nitrogen sampling and analysis

The mean level of leaf nitrogen for each tree was calculated each month by taking six leaves within the same age class as those leaves used to measure the rate of herbivory. Leaves were dried at 80°C for 48 h, ground in a Wiley mill and analysed for nitrogen content using the Kjeldahl technique (Nelson and Sommers, 1980).

Statistical analyses

To examine the possible inter-relationships between the herbivory rate, the number of herbivores on trees and leaf nitrogen content, a series of analyses of covariance (ANCOVA) were performed. These estimated regression coefficients were standardized for individual month and tree effects. Analyses of covariance were performed using the MANOVA least-squares procedures of the SPSSX statistical package (SPSS Inc., 1986). In the case of *E. marginata*, a nested ANCOVA was performed. During the analyses, nitrogen levels were assigned to the previous month's level of herbivory, as leaf traces were made on the same day as the leaves for nitrogen content analysis were picked.

RESULTS

Herbivore abundance

The four most common orders of herbivorous insects collected during the study, in order of abundance, were Coleoptera, Hemiptera, Collembola and Lepidoptera. The numbers of insects in the orders Coleoptera and Hemiptera were lowest during the winter (July, August), gradually increased to reach a peak in early summer (December) and decreased towards the end of summer (Fig. 1(a),(c)). The Collembola and Lepidoptera had fewer individuals overall and a less seasonal variation in the number of herbivores than the Coleoptera and Hemiptera (Fig. 1(b),(d)). Peak numbers of herbivorous Collembola and Lepidoptera occurred in October and September, respectively.

Herbivore abundance was lowest in the early months of winter, gradually increased to reach a peak in early summer and then decreased towards the end of summer (Fig. 2). Overall, the greatest number of herbivores occurred on *E. accedens*. This was closely followed by *E. marginata* in the forest and *E. resinifera*. *Eucalyptus marginata* on the minesite had the lowest overall number of herbivores throughout the study. The abundance of herbivores

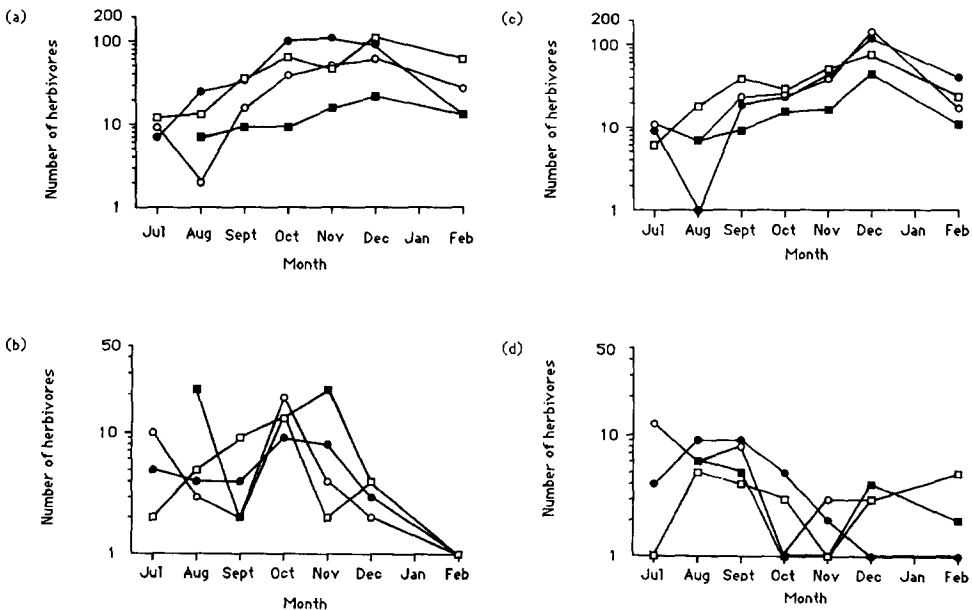


Fig. 1. Herbivore abundance for the orders; (a) Coleoptera, (b) Collembola, (c) Hemiptera and (d) Lepidoptera, on the species (●) *Eucalyptus accedens*, (○) *E. resinifera*, (■) *E. marginata* (minesite) and (□) *E. marginata* (forest).

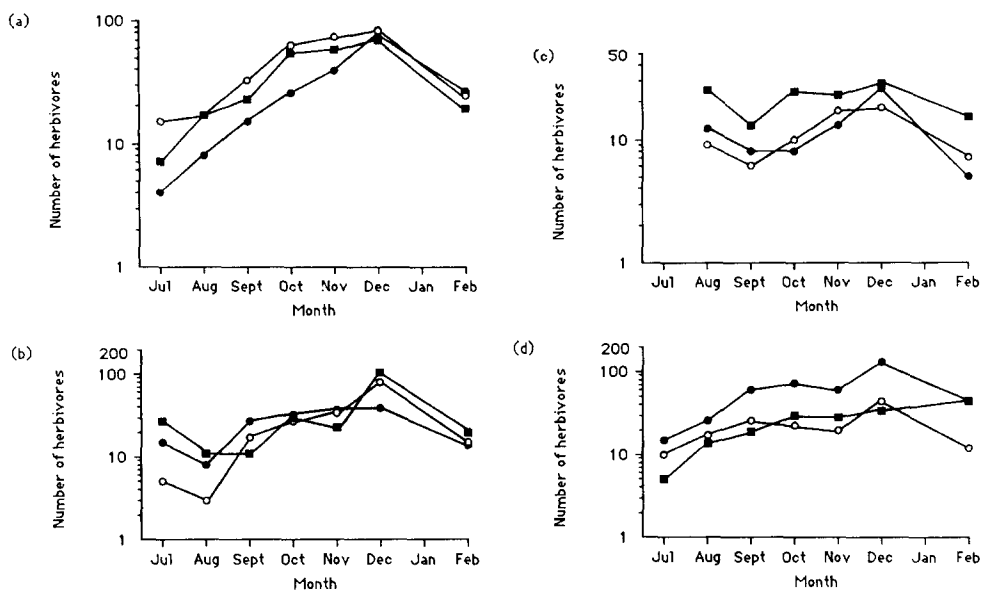


Fig. 2. The number of herbivores collected on three different trees of (a) *Eucalyptus accedens*, (b) *E. resinifera*, (c) *E. marginata* (minesite) and (d) *E. marginata* (forest).

varied significantly in *E. accedens* ($P < 0.001$), *E. marginata* on the minesite ($P < 0.001$) and *E. marginata* in the forest ($P < 0.01$).

The rate of herbivory

The mean monthly rate of herbivory by insects varied from 0 to 3.3% (Fig. 3). The greatest mean monthly leaf loss was 1.8 (± 0.3)% in *E. marginata* growing on the minesite, followed by *E. resinifera* with 1.2 (± 0.3)%, *E. marginata* in the forest with 1.1 (± 0.2)% and *E. accedens* with 0.9 (± 0.2)%. These rates of leaf loss were significantly different between eucalypt species and between *E. marginata* on the minesite and in the regenerated forest ($P < 0.05$).

Leaf nitrogen

The level of leaf nitrogen in eucalypts varied from 0.8 to 2.0% of dry weight (Fig. 4). *Eucalyptus resinifera* had the highest mean monthly level of leaf nitrogen with 1.43 (± 0.05)%, followed by *E. accedens* with 1.40 (± 0.03)%, *E. marginata* in the forest with 1.15 (± 0.03)% and *E. marginata* on the minesite with 0.99 (± 0.03)%. There was a significant difference in the level of leaf

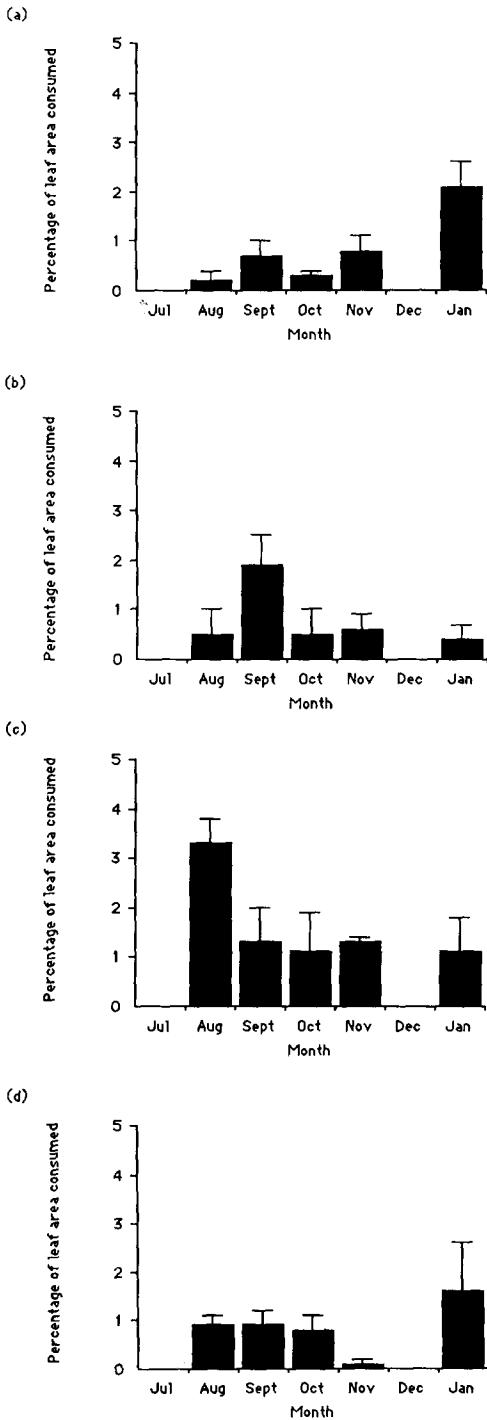


Fig. 3. Mean (\pm S.E.) percentage of herbivory on (a) *Eucalyptus accedens*, (b) *E. resinifera*, (c) *E. marginata* (minesite) and (d) *E. marginata* (forest).

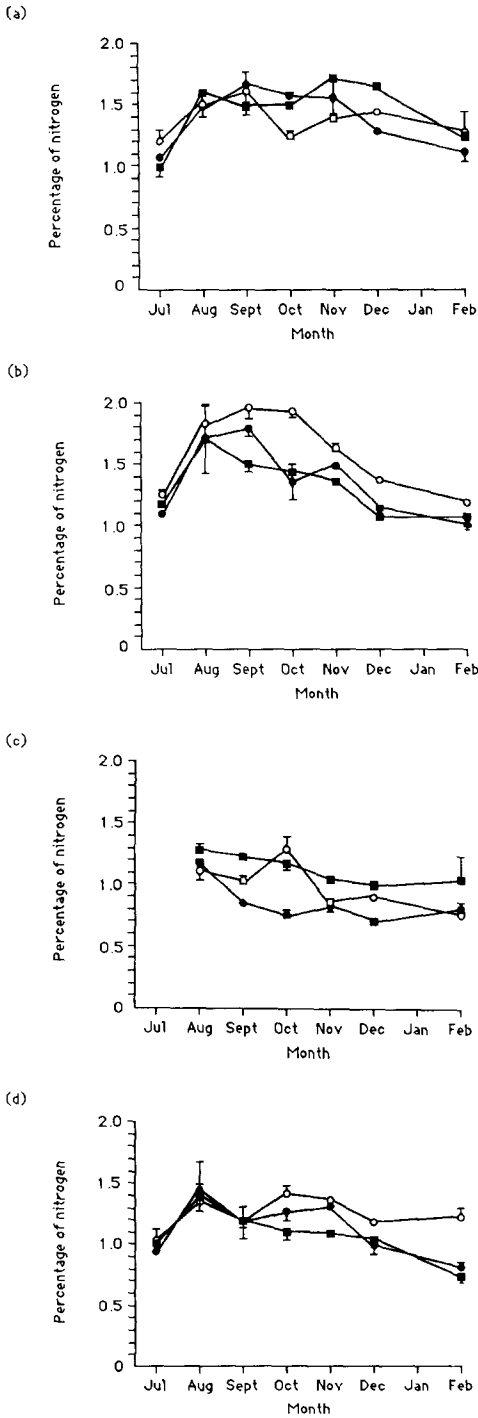


Fig. 4. The mean (\pm S.E.) amount of leaf nitrogen on three trees of (a) *Eucalyptus accedens*. (b) *E. resinifera*, (c) *E. marginata* (minesite) and (d) *E. marginata* (forest).

nitrogen in individual trees of *E. resinifera* ($P < 0.001$) and *E. marginata* in the forest ($P < 0.05$). The monthly variations in leaf nitrogen levels were highly significant ($P < 0.001$) except in *E. marginata* on the minesite.

An analysis of covariance with herbivore abundance as the dependent variable, revealed that in *E. accedens* the level of nitrogen in the previous month was positively correlated ($P < 0.05$) to the following month's level of herbivore abundance. In *E. resinifera*, the current month's level of leaf nitrogen showed a significant negative association ($P < 0.05$) with the number of herbivores on the tree.

In *E. accedens* the previous month's level of leaf nitrogen and abundance of herbivores showed a significant positive regression ($P < 0.01$) and ($P < 0.001$), respectively, with the rate of herbivory. The current month's nitrogen showed a negative regression ($P < 0.05$) with the rate of herbivory. In *E. resinifera*, the previous month's level of nitrogen showed a significant positive regression with the rate of herbivory. In *E. marginata* the current and previous month's level of leaf nitrogen showed a positive regression ($P < 0.05$) with the rate of herbivory.

The rate of herbivory was negatively correlated ($P < 0.05$) to the following month's level of leaf nitrogen as were the current and previous month's level of herbivore abundance ($P < 0.001$) in *E. resinifera*.

DISCUSSION

Low levels of nitrogen in the leaves of plants, have been associated with a low growth rate of herbivorous insects (Feeny, 1970). Since plants use defences such as increasing the level of leaf tannins and the toughness of leaves to deter predation (Feeny, 1976), a decrease in the level of nitrogen could possibly represent a defence strategy in plants. As this study did not monitor the level of secondary compounds, the authors do not suggest that the rate of herbivory was solely affected by changes in the level of leaf nitrogen or other factors that may be strongly correlated with nitrogen content, but simply that variations in these levels may have influenced the rate of herbivory. Furthermore, the regressions between herbivory rate, herbivory abundance and leaf nitrogen levels were not consistently significant in all the eucalypts studied. This would suggest that factors other than nitrogen had an effect on the amount of leaf material removed by herbivores. During this study, Coleoptera and Hemiptera were the most numerous herbivores collected, a result also found by Lowman (1982), and these orders are probably the dominant herbivores on eucalypt leaves. The Coleoptera and the Lepidoptera (larvae) have biting mouthparts and probably contributed to removing a greater quantity of leaf material than the Hemiptera and Collembola which have sucking mouthparts.

The hypothesis that eucalypts may lower leaf nitrogen in response to a high rate of leaf consumption, suggests that firstly, a high level of leaf nitrogen

should be associated with a high level of herbivory in the same and in the following month. Secondly, that a low level of leaf nitrogen should tend to be associated with a high level of herbivory in the previous month. In addition, if the count of insect numbers reflects the damage caused by those insects, then we should expect a high rate of herbivory to be associated with a high insect abundance and the pattern of change of the insect counts to reflect that of the herbivory rate.

There was a significant positive regression of the average leaf nitrogen level for the current month on the herbivory rate in *E. marginata*, and a significant positive regression of the previous month's average nitrogen in all three species. This indicates strong support for the first relationship discussed above. Previous studies have shown the same trend in both eucalypt (Fox and Macauley, 1977; Landsberg and Wylie, 1983) and non-eucalypt species (McNeill and Southwood, 1978; Onuf, 1978; Mattson, 1980; Mooney and Gulmon, 1982).

A significant negative regression of leaf nitrogen in the preceding month's herbivory rate was found in *E. resinifera*, but not found in the other two species. Thus, the second relationship is supported by only one species under study. However, there is little support for the third relationship, that insect abundance should vary with the herbivory rate. Insect abundance represents purely a count of numbers obtained from combining two sampling techniques, and may not reflect the consumption activity of the insects for a variety of reasons. Despite this, insect abundance did behave as expected in *E. resinifera*.

Nitrogen levels in plants vary with seasons (Edwards and Wratten, 1980) and with natural conditions which cause stress within plants (White, 1984). The correlations between the rate of herbivory, herbivore abundance and the level of leaf nitrogen found in *E. resinifera*, suggest that some eucalypts may lower the level of leaf nitrogen in response to an increase in predation. Fox and Macauley (1977) however, found no evidence to suggest that grazers have the ability to alter feeding rates in eucalypts with low levels of nitrogen. However, in this study, factors other than nitrogen might have influenced the level of herbivory. Previous studies have indicated that the moisture, fibre content and amount of secondary compounds in the leaves also play an important role in regulating the amount of predation by insect herbivores.

ACKNOWLEDGEMENTS

We would like to acknowledge the help of P. Arena, I. Bennett, M. Lund, D. Plaskett and M. Ramsey for advice and assistance in the field. Thanks must also be given to the Biology Teaching Preparations staff at Murdoch University, for their kind use of equipment and facilities. Funds for this study were provided by Alcoa of Australia and Murdoch University.

REFERENCES

- Bray, J.R., 1961. Measurement of leaf utilization as an index of minimum level of primary production. *Oikos*, 12: 70–74.
- Bray, J.R., 1964. Primary consumption in three forest canopies. *Ecology*, 45: 165–167.
- Carne, P.B., Greaves, R.T.G. and McInnes, R.S., 1974. Insect damage to plantation-growth eucalypts in north coastal New South Wales, with particular reference to Christmas beetles (Coleoptera:Scarabaeidae). *J. Aust. Entomol. Soc.*, 13: 189–206.
- Coley, P.D., 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol. Monogr.*, 53: 209–233.
- Crawley, M.J. (Editor), 1983. Plant–herbivore dynamics. In: *Herbivory: The Dynamics of Animal–Plant Interactions*. Blackwell Scientific Publications, London, pp. 211–289.
- Edwards, P.J. and Wratten, S.D. (Editors), 1980. Defense mechanisms. In: *Ecology of Insect–Plant Interactions*. Edward Arnold, London, pp. 30–32.
- Elzinga, R.J. (Editor), 1978. *Fundamentals of Entomology*. Prentice–Hall, New Jersey.
- Feeny, P., 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology*, 51: 565–581.
- Feeny, P., 1976. Plant apparency and chemical defences. *Rec. Adv. Phytochem.*, 10: 3–40.
- Fox, L.R. and Macauley, B.J., 1977. Insect grazing on *Eucalyptus* in response to variation in leaf tannins and nitrogen. *Oecologia*, 29: 145–162.
- Fox, L.R. and Morrow, P.A., 1983. Estimates of damage by herbivorous insects on *Eucalyptus* trees. *Aust. J. Ecol.*, 8: 139–147.
- Fox, L.R. and Morrow, P.A., 1986. On comparing herbivore damage in Australia and northern temperate systems. *Aust. J. Ecol.*, 11: 387–393.
- Journet, A.R.P., 1981. Insect herbivory on the Australian woodland eucalypt, *Eucalyptus blakelyi* M. *Aust. J. Ecol.*, 6: 135–138.
- Kile, G.A., 1974. Insect defoliation in the eucalypt regrowth forests of southern Tasmania. *Aust. For. Res.*, 6: 9–18.
- Landsberg, J. and Wylie, F.R., 1983. Water stress, leaf nutrients and defoliation: a model of dieback in rural eucalypts. *Aust. J. Ecol.*, 8: 27–41.
- Lowman, M.D., 1982. Seasonal variation in insect abundance among three Australian rainforests, with particular reference to phytophagous types. *Aust. J. Ecol.*, 7: 353–361.
- Mattson, W.J., Jr., 1980. Herbivory in relation to plant nitrogen content. *Annu. Rev. Ecol. Syst.*, 11: 119–161.
- Mattson, W.J. and Addy, N.D., 1975. Phytophagous insects as regulators of forest primary production. *Science*, 190: 515–521.
- McNaughton, S.J., 1979. Grazing as an optimizing process: grass–ungulate relationships in the Serengeti. *Am. Nat.*, 113: 691–703.
- McNeill, S. and Southwood, T.R.E., 1978. The role of nitrogen in the development of insect/plant relationships. In: J.B. Harborne (Editor), *Biochemical Aspects and Animal Co-evolution*. Academic Press, London, pp. 77–98.
- Mooney, H.A. and Gulmon, S.L., 1982. Constraints on leaf structure and function in reference to herbivory. *BioScience*, 32: 198–206.
- Morrow, P.A., 1977. Host specificity in a community of three co-dominant *Eucalyptus* species. *Aust. J. Ecol.*, 2: 89–106.
- Morrow, P.A., 1983. The role of sclerophyllous leaves in determining insect grazing damage. In: F.J. Kruger, D.T. Mitchell and J.U.M. Jarvis (Editors), *Mediterranean-type Ecosystems*. Springer-Verlag, Berlin, pp. 509–519.
- Nelson, D.W. and Sommers, L.E., 1980. Total nitrogen analysis of soil and plant tissues. *J. Assoc. Off. Anal. Chem.*, 63: 770–777.

- Onuf, C.P., 1978. Nutritive value as a factor in plant-insect interactions with an emphasis on field studies. In: G.G. Montgomery (Editor), *The Ecology of Arboreal Folivores*. Smithsonian Institution Press, Washington, pp. 85-96.
- Onuf, C.P., Teal, J.M. and Valiela, I., 1977. Interactions of nutrients, plant growth and herbivory in a mangrove ecosystem. *Ecology*, 58: 514-526.
- Reichle, D.E., Goldstein, R.A., Van Hook, Jr., R.I. and Dodson, G.J., 1973. Analysis of insect consumption in a forest canopy. *Ecology*, 54: 1076-1084.
- Rodriguez, J.G., 1960. Nutrition of the host and reaction to pests. *Pub. Am. Assoc. Adv. Sci.*, 61: 149-167.
- Slansky, F. and Feeny, P., 1977. Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. *Ecol. Monogr.*, 47: 209-228.
- Soo Hoo, C.F. and Fraenkel, G., 1966a. The selection of food plants in a polyphagous insect, *Prodenia eridania* (Cramer). *J. Insect Physiol.*, 12: 693-709.
- Soo Hoo, C.F. and Fraenkel, G., 1966b. The consumption, digestion and utilization of food plants by a polyphagous insect, *Prodenia eridania* (Cramer). *J. Insect Physiol.*, 12: 711-730.
- Springett, B.P., 1978. On the ecological role of insects in Australian eucalypt forests. *Aust. J. Ecol.*, 3: 129-139.
- SPSS Inc., 1986. *SPSS Users Guide*. SPSS Inc., Chicago, IL, 2nd edn., 988 pp.
- Tuomi, J., Niemela, P., Haukioja, E., Siren, S. and Neuvonen, S., 1984. Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. *Oecologia*, 61: 208-210.
- Wareing, P.F., 1970. Growth and its coordination in trees. In: L.C. Luckwill and C.V. Cutting (Editors), *Physiology of Tree Crops*. Academic Press, New York, NY, pp. 1-21.
- White, T.C.R., 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia*, 63: 90-105.