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PATTERNS OF BIOACTIVITY AND HERBIVORY ON *Nothofagus* SPECIES FROM CHILE AND NEW ZEALAND

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Abstract—*Nothofagus* species from Chile and New Zealand were surveyed in the field for invertebrate abundance and leaf feeding damage and in the laboratory for antifeedant activity against leafrollers (*Ctenopsteustis obliquana*, *Epiphyas postvittana*), deterrent activity against pea aphid (*Acyrthosiphon pisum*), insect growth regulatory activity (*Oncopeltus fasciatus*), nematicidal activity (*Caenorhabditis elegans*), antibiotic activity (*Pseudomonas solanaciarium*), and general toxicity. A data matrix indicated that *N. alessandri* and *N. pumilio* most likely have a chemical barrier to insect attack as leaves showed low faunal abundance, low herbivory, and activity in the leafroller antifeedant, aphid deterrent, and nematicidal assays. A chemical examination of *N. alessandri* that used the leafroller antifeedant test to guide the separation yielded an active fraction containing the flavonoid, galangin, and the stilbene, pinosylvin, which appear to act in concert to deter leafroller feeding. The discovery of the phytoalexin, pinosylvin, in the leaves, raises the possibility that *Nothofagus* in general, and *N. alessandri* in particular, may have induced chemical defense mechanisms.

Key Words—*Nothofagus*, faunal abundance, herbivory, bioactivity, leafroller antifeedant activity, aphid deterrency, pinosylvin, galangin, *Ctenopsteustis obliquana*, *Epiphyas postvittana*, *Acyrthosiphon pisum*.

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INTRODUCTION

The genus *Nothofagus* (Fagaceae) comprises about 35 species distributed in South America, Australia, New Zealand, New Caledonia, and Papua New Guinea. This disjunct distribution represents a canonical example of biogeographical patterns arising from the continental drift hypothesis (Poole, 1987). Furthermore, its wide distribution and the restriction of subgenera to different regions make it eminently suitable for a comparative study of the biological and chemical characteristics that may have arisen as the disparate populations continued to evolve in the face of selection pressures by invertebrates.

McQuillan (1993) has reviewed what is known of the invertebrate fauna associated with *Nothofagus* and the role it plays in the ecology of forests. Generally, *Nothofagus* supports about 30 genera, many of which are specialized on the host plant, reflecting the long history of the genus. Sap-sucking Homoptera are particularly diverse and host-specific, whereas defoliating insects seem less important and specific. However, while it is unlikely that there has been a tight stepwise, coevolutionary mechanism for reciprocal change and adaptations among the interacting species, it is clear that *Nothofagus* has responded continually to many invertebrate species in ecological and evolutionary time. There are certain features of *Nothofagus* that can be attributed to antiherbivore roles. Tough coriaceous leaves, hard seed capules, and the presence of phenolic compounds and tannins all indicate a generalized resistance to insect feeding of a type expected for highly apparent, long-lived plants (Feeny, 1976; Rhodes and Cates, 1976; Fox, 1981).

There have been some phytochemical studies on *Nothofagus*. Hillis and Orman (1962) reported the isolation of several flavonoids from heartwood of the New Zealand species, showing there is considerable interspecific variation between wood and leaves, and Wollenweber et al. (1997) found several flavonoids and glycosides in the leaf exudates of *N. antarctica*. A recent report (Quiroz et al., 1999) identifies the volatiles emitted from the leaves of six Chilean *Nothofagus* species and compares a major terpene constituent, α-agarofuran, with patterns of aphid-host specificity. Lowman and Box (1983), in herbivory studies on *N. moorei*, showed that, in general, leaf toughness and toxicity (phenolics and tannins) increased as the leaves aged and that corresponding insect grazing decreased. Despite these studies, the data on *Nothofagus* are sporadic and patchy. Although there have been many studies with New Zealand and Australian *Nothofagus*, particularly with respect to invertebrate associations, data from South America are uneven and from New Caledonia and New Guinea, nonexistent. In order to understand if chemical defense is an important parameter in *Nothofagus*–insect ecology, basic data are needed on the occurrence and role of individual bioactive metabolites (Berenbaum, 1995), their distribution within the plant and the genus, and their dynamics and correlation with herbivory.

In this study, we have surveyed the Chilean and New Zealand *Nothofagus* for invertebrate fauna and associated leaf damage, and by using established laboratory bioassays we have sought bioactivity in the leaf extracts. In this way, we hoped to establish if any species is better defended against insect herbivores, which could then lead into more detailed chemical studies on the type and nature of these characteristics as a first step in delineating and comparing the chemical ecology within the genus.

METHODS AND MATERIALS

Study Sites. The collection of plant material and a survey of extant insects on *Nothofagus* in Chile and New Zealand were carried out at selected sites during December 1995 and February 1996. The sites in Chile were: Altos de Lircay National Reserve for *N. glauca*, *N. obliqua*, *N. dombeyi*, *N. pumilio*, and *N. alpina*; Cerro el Roble National Reserve for *N. obliqua* var. *macrocarpa*; Los Ruiles National Reserve for *N. alessandri* and *N. leoni*; Nulhuelbuta National Park for *N. antarctica*; and Puyehue National Park for *N. betuloides* and *N. nitida*. The sites in New Zealand were: Mt Murchison Forest Reserve for *N. solandri*, *N. fusca*, *N. menziesii*, and *N. solandri* var. *cliffortoides*; and Mareti Valley, Nelson, for *N. truncata*.

Insect Collection. Invertebrate fauna was collected from five trees of each species located in close proximity. For each tree, a branch 3–4 m from the ground was shaken vigorously, and the invertebrates were collected on a canvas tray and stored in vials containing 70% ethanol.

Plant Collection. Leaves from each species were collected and air-dried. Plant material (10 g) was milled and extracted sequentially with dichloromethane (100 ml) and methanol (100 ml), and the solvent evaporated to give two residues per plant. Leaves (100) were randomly selected from insect-sampled trees and visually assessed for the percent (to the nearest unit of 5) of damaged leaf area. The data were tested for normality and then subjected to an ANOVA and Tukey analysis.

Antifeedant Test. The two-way choice test design of Blaney et al. (1990) was used with two New Zealand insects, the brown-headed leafroller (*Ctenopsteustis obliquana*) and the light-brown apple moth (*Epiphyas postvittana*). Fifth-instar larvae were presented with diet squares incorporating combined dichloromethane and methanolic extracts of the plant material to make a final concentration of 1% w/w plant extract on the moistened square (Russell and Lane, 1993). Antifeedant activity was assessed by recording the weight of test square eaten after 24 hr compared to a control square. A feeding index ($FI = [(C-T)/(C+T)] \times 100$) was calculated from the amount eaten of the control (C) and test (T) squares, respectively. An antifeedant extract has a positive index; a

negative index indicates feeding stimulation. The treatment versus control data of 10 replicates were analyzed by the Wilcoxon matched-pairs test.

The diet squares were prepared by spreading hot standard rearing diet (Singh, 1983) on 20 × 20-cm glass plates with a thin-layer spreader set at 1-mm thickness, cutting the cooled diet into 15-mm squares, and freeze drying. The squares were moistened with 70 μl of water before presentation to the larvae and dried in a desiccator after the feeding test.

Aphid Settling and Reproduction. Leaves (leaflet pairs, ca. 0.3 g) from young bean plants (*Phaseolus vulgaris* cv. Tendergreen) were treated with a combined dichloromethane and methanolic plant extract dissolved in acetone (1% w/w per leaf). The acetone solution was applied to both surfaces. Control leaves were treated with acetone only. Each leaflet pair was placed through a hole in a plastic Petri dish so that the petiole protruded into distilled water in the dish. Ten apterous adults of the pea aphid (*Acyrthosiphon pisum*) were placed on the lid of each Petri dish (treated and control), and then a treated and control dish were placed together in a plastic cage. There were six replicates. After 24 hr, the position of the aphids within the cage was recorded by counting the numbers of adults that had moved onto either the treated or control leaves. The number of nymphs that had been produced on each leaflet pair within each cage was also recorded. The Fisher least significant test was used to analyze aphid settling and reproduction, while comparison among the plant extracts was made by using the "matrix of pairwise" test.

Cytotoxicity. A standard cytotoxicity test was carried out with cultures of KB cells (Mosman, 1983). Each plant extract was dissolved in dimethyl sulfoxide to make a final concentration in the cell suspension of 10 μg/ml. After three days, the surviving cells were assessed and compared with adriblastine as a cytotoxic control.

Insect Growth Regulation. A glass Petri plate was coated with 100 μl of an acetone solution of each plant extract, and the solvent was allowed to evaporate so that the dish was coated at 80 μg/cm^2. Twenty milkweed bugs (2nd instar, *Oncopeltus fasciatus*) were added to each dish together with food (sunflower seeds) and water and allowed to develop to maturity (14 days). Insects were observed for mortality, lack of growth, and abnormal development including juvenilization and antijuvenile hormone activity through having contact with the plant extracts (Bowers, 1968).

Nematicidal Assay. Plant extract (0.5 g) was added to a suspension of nematodes (*Caenorhabditis elegans*, 500–1000/ml) in M-9 buffer (0.5 ml) (Evans et al., 1984). Nematodes surviving after 24 hr were assessed under a dissecting microscope.

Invertebrate Toxicity. As a general test for invertebrate toxicity, brine shrimp (*Artemia salina*) were used in the standard test of Meyer et al. (1982). To each plant extract (50 μg) were added 20 newly hatched shrimp in brine (0.5 ml). Mortality of the shrimp was assessed after 24 hr.

Antibiotic Activity. Plant extracts (0.5 mg) in solvent were absorbed into filter paper disks (0.6 cm), and the air-dried disks were placed onto an agar surface that had been uniformly coated with a suspension of *Pseudomonas solanaciarium* in water. The plates were incubated for 24 hr at room temperature when a lawn of bacteria covered the agar surface, except for zones of growth inhibition around disks with bactericidal activity.

Extraction of N. alessandri. Dried ground leaves of *N. alessandri* (20 g) were extracted with AR methanol, filtered, and the solvent removed to give a residue (5 g) that had antifeedant activity in the two-way choice test with *Ctenopsteustis obliquana*. The residue was partitioned between methanol–water (4 : 1, 100 ml) and hexane (100 ml), and each phase was repartitioned with fresh solvent. The combined methanolic and hexane phases were evaporated to dryness. The residue (3 g) from the methanolic phases retained the antifeedant activity. This was subjected to flash chromatography on a column of silica gel (Merck 60H) tightly packed into a column (3 cm diam.) and eluted with mixtures of dichloromethane–methanol (50 ml, DCM, DCM/MeOH, 19 : 1, 9 : 1, 4 : 1, MeOH). The antifeedant activity was found in the fraction eluting with DCM/MeOH, 19 : 1. This fraction contained two main components, which were separated by chromatography on Sephadex LH_{20} by eluting with DCM/MeOH 19 : 1, and identified as galangin (**1**) and pinosylvin (**2**) (Figure 1). The DCM fraction from the flash chromatography was also chromatographed on Sephadex LH_{20} to give galangin, 8-methoxygalangin (**3**), and 3-*O*-methyl-8-methoxygalangin (**4**) (Figure 1).

Galangin (***1***). Crystals from EtOH, 20 mg, mp 214–215°C. UV: λ (MeOH) 358, 310, 266 nm, λ (MeOH + $AlCl_3$) 412, 336, 304, 272 nm. ^{1}H NMR (300 MHz, d-$CHCl_3$): δ12.08 (brs, 1H, 5-OH), 8.26 (d, 2H, C-2′ and C-6′), 7.54 (m, 3H, C-3′, C-4′, C-5′), 6.56 (s, 1H, C-8), 6.30 (s, 1H, C-6). EI-MS (*m*/*z*): 270, 242, 213, 198, 168, 153, 146, 105, 78; $C_{15}H_{10}O_5$ requires 270.04738, found 270.047485.

Pinosylvin (***2***). 36 mg, mp 155–156°C. UV: λ (MeOH) 308, 300 nm. ^{1}H NMR (300 MHz, d-$CHCl_3$): δ8.3 (brs, 1H, OH), 7.57 (m, 2H), 7.30 (m, 3H), 7.09 (s, 2H), 6.60 (d, 2Hz, 2H), 6.33 (tr, 1H). EI-MS (*m*/*z*): 212, 197, 165, 141, 128; $C_{14}H_{12}O_2$ requires 212.083730, found 212.083324.

8-Methoxygalangin (***3***). 25 mg, mp 176–178°C. UV: λ (MeOH) 365, 308, 295, 270 nm, λ (MeOH + $AlCl_3$) 423, 341, 311, 275 nm. ^{1}H NMR (300 MHz, d-$CHCl_3$): δ11.79 (brs, 1H, 5-OH); 8.31 (d, 2H, C-2′ and C-6′), 7.58 (m, 3H, C-3′, C-4′, C-5′), 6.34 (s, 1H, C-6), 3.97 (s, 3H, OMe). EI-MS (*m*/*z*): 300, 285, 157, 139, 105, 77; $C_{16}H_{12}O_6$ requires 300.063388, found 300.063904.

3-O-*Methyl-8-methoxygalangin* (***4***). 10 mg, mp 170–175°C. UV: λ (MeOH) 354, 272 nm, λ (MeOH + $AlCl_3$) 412, 383, 286 nm. ^{1}H NMR (300 MHz, d-$CHCl_3$): δ12.38 (brs, 1H, 5-OH), 8.16 (d, 2H, C-2′ and C-6′), 7.58 (m, 3H, C-3′, C-4′, C-5′), 6.34, (s, 1H, C-6), 3.92 (s, 3H, OMe), 3.90 (s, 3H, OMe), 3.90

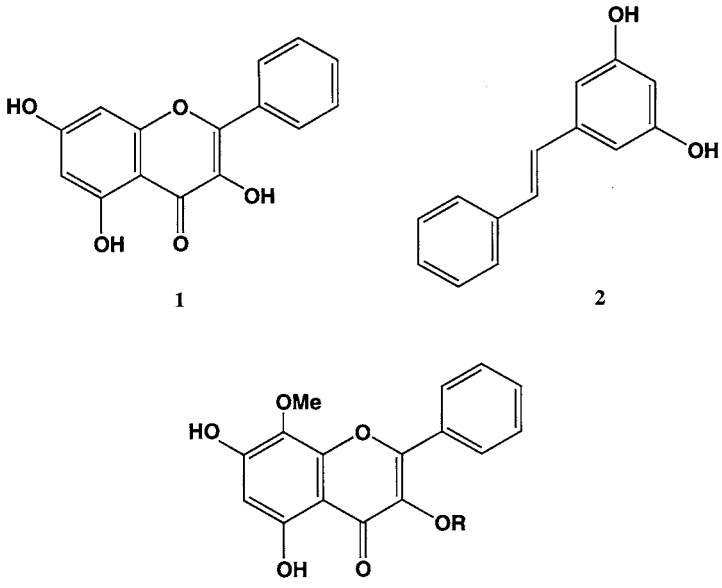

FIG. 1. Structures of compounds **1–4**.

(s, 3H, OMe). EI-MS (*m/z*): 314, 199, 186, 171, 182, 167, 139, 118, 105, 77, 69; $C_{17}H_{14}O_6$ requires 314.079038, found 314.077271.

RESULTS AND DISCUSSION

The arthropod survey, which was conducted over a short time frame, was compared to the comprehensive analysis of *Nothofagus* invertebrate fauna made by McQuillan (1993), in order to assess how representative the survey actually was. The sum, over all *Nothofagus* species, of the number of recognizable taxonomic units (RTUs) in each insect order was higher in the present survey (560 RTUs) than in McQuillan's review (339 RTUs, Table 1). The differences between the two sets of data may be the result of the present survey (1) including predatory RTUs, (2) not distinguishing if the RTUs are merely "tourist" species rather than being closely associated with the trees, and (3) being severely limited in space and time. For example, the greater diversity of Hymenoptera in the present survey (59 vs. 5) may reflect the inclusion of predatory RTUs, whereas the Lepidoptera, which have insignificant predatory taxa, showed simi-

TABLE 1. INVERTEBRATE FAUNA ASSOCIATED WITH NOTHOFAGUS[a]

Nothofagus species	Total		Coleoptera		Homoptera		Lepidoptera		Diptera		Hymenoptera		Heteroptera	Spiders	Other	
	TS	McQ	TS	McQ	TS	McQ	TS	McQ	TS	McQ	TS	McQ	(TS)	(TS)	TS	McQ
obliqua	103	38	29	16	8	13	13	10	5	1	17	1	3	19	9	0
antarctica	64	28	18	12	5	9	18	5	1	0	4	1	3	9	6	3
solandri	64	37	15	5	2	17	13	14	11	0	5	0	1	12	5	2
alpina	56	6	9	4	6	1	5	0	3	0	4	1	3	18	3	0
betuloides	53	8	7	1	5	7	10	0	6	0	5	0	4	12	4	0
pumilio	51	19	16	10	4	3	4	5	5	0	4	1	5	10	3	0
solandri var. *cliff.*	51	35	9	5	2	14	8	16	11	1	6	0	1	9	5	2
dombeyi	48	55	15	35	8	13	6	5	3	0	2	1	4	7	3	1
leoni	45	0	5	0	6	0	3	0	0	0	4	0	0	22	5	0
menziesii	38	54	13	6	1	23	7	23	7	0	2	0	0	6	0	9
nitida	36	1	9	0	5	1	5	0	1	0	1	0	1	10	4	0
fusca	34	37	8	3	1	19	15	14	4	0	1	0	1	3	2	5
alessandri	31	0	4	0	4	0	0	0	2	0	0	0	1	17	3	0
truncata	28	19	10	3	1	2	7	15	1	0	1	0	0	3	5	2
glauca	22	2	2	1	4	1	0	0	0	0	3	0	1	7	5	0
Total	724	339	169	101	62	123	114	107	60	2	59	5	28	164	62	24

[a]A comparison of recognizable taxonomic units (RTUs) found in this survey (TS) with those from previous surveys, as analyzed by McQuillan (1993) (McQ). Species are listed in order of faunal diversity (from greatest to least).

TABLE 2. INVERTEBRATE ABUNDANCE AND CONTRIBUTION OF INSECT GUILDS TO FAUNA ASSOCIATED WITH *Nothofagus*

Nothofagus species[a]	Total Abundance[b]	Chewer (%)	Sucker (%)	Predator (%)	Other (%)
antarctica	553	68	14	12	6
obliqua	512	15	35	33	17
pumilio	490	11	63	17	9
leoni	238	19	17	59	5
solandri var. *cliff.*	230	11	4	30	55
betuloides	217	11	41	36	12
solandri	205	34	3	49	14
alpina	182	25	11	51	13
dombeyi	158	17	23	46	14
menziesii	124	60	2	15	23
fusca	112	67	1	23	9
nitida	101	19	17	43	21
truncata	75	36	2	39	23
alessandri	64	17	19	58	6
glauca	54	13	11	52	24

[a]Species are listed in order of faunal abundance (from greatest to least).
[b]Abundance figures represent the total numbers of individual invertebrates collected from beatings. The figures represent sampling of five trees in one location per species.

lar numbers of RTUs (114 vs. 107). On the other hand, although the Coleoptera would also be expected to have few predatory RTUs, the present survey recorded greater diversity than the review (169 vs. 107), and there may be some tourist species included in this order. The differences in Diptera RTUs (60 vs. 2) can be attributed to the tourist nature of most of this order in our survey. Finally, the Homoptera yielded a significantly lower number of RTUs in the present survey as compared with the review (62 vs. 123). The greatest difference stems from the survey of the New Zealand species (*N. menziesii*, *N. fusca*, *N. truncata*, *N. solandri*, and *N. solandri* var. *cliffortioides*, 7 vs. 75). Most of the Homoptera reported for the New Zealand species are coccids whose subtle interspecific differences will not have been apparent in our survey. In spite of its narrowness in space and time, our survey may be taken as a representative cross section of the invertebrate fauna associated with *Nothofagus*.

The total abundance and the relative abundance of different insect guilds in each *Nothofagus* species are presented in Table 2. *N. antarctica*, *N. obliqua*, and *N. pumilio* showed the greatest faunal abundance, and *N. glauca*, *N. alessandri*, and *N. truncata*, the least. Figure 2 shows that abundance and diversity are positively correlated. Points for *N. pumilio* and *N. antarctica* were considered outliers since they fell outside the 95% confidence interval. The Spearman rank order correlation without these two points gave $R = 0.857$, $P < 0.001$. The data

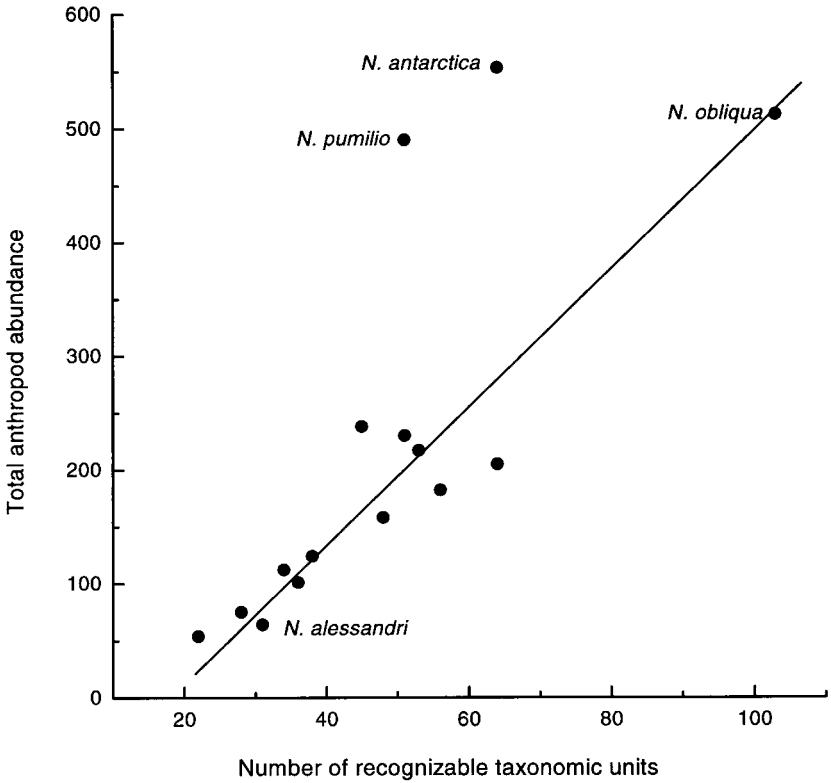

FIG. 2. Correlation between diversity and abundance of arthropods on *Nothofagus* species. The line excludes the coordinates for *N. antarctica* and *N. pumilio*.

suggest a faunal distribution less uniform in *N. pumilio* and *N. antarctica* than in the other species.

The damage assessment of leaves from all species is given in Figure 3. Not surprisingly, feeding damage was correlated with the abundance of chewing insects (Spearman rank order correlation, $R = 0.561$, $P = 0.029$). *N. antarctica* and *N. obliqua* showed the greatest leaf damage, while *N. solandri* var. *cliffortoides*, *N. truncata*, and *N. alessandri* showed the least.

The ecological survey has given results that quantify the extent of insect herbivory on *Nothofagus* in a specific space and time frame. This is the first time that all species of South American *Nothofagus* have been examined systematically and in comparison with each other and with the New Zealand species. The data are not comprehensive in that they do not represent all insects that may prefer *Nothofagus* as host plant, but it does represent a window on the associated

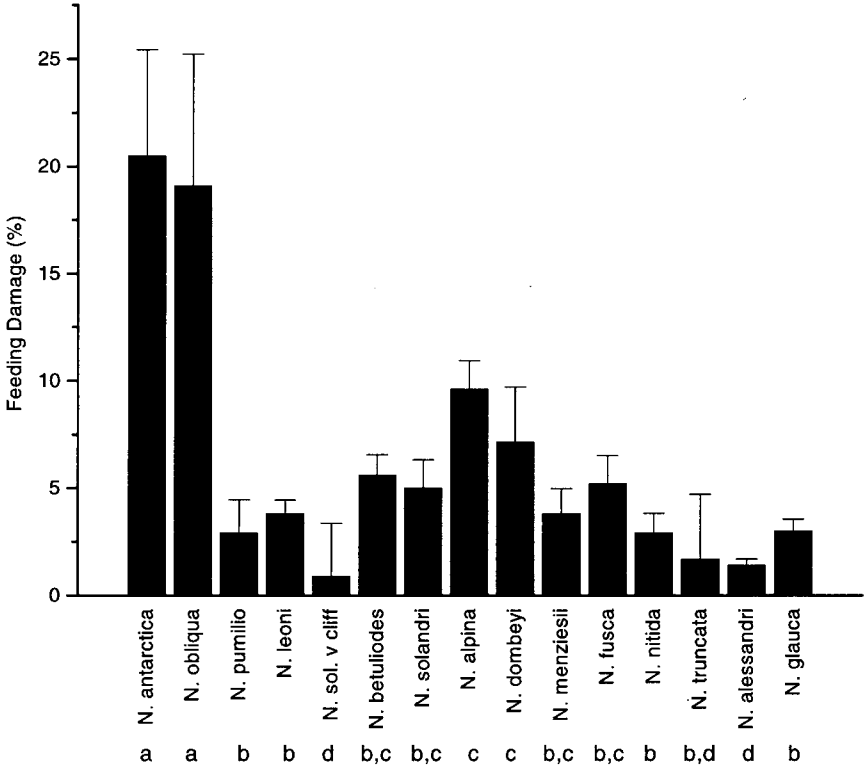

FIG. 3. Feeding Damage (% of leaf consumed) of *Nothofagus* leaves. Species are ordered from the greatest to least faunal abundance as found in the survey (Table 2). Similarities and differences (Tukey) are indicated by common or different letters, respectively ($P < 0.05$).

insects and the extent of herbivory in a particular point in space and time. Once the chemistry of a particular species is delineated, a detailed herbivory study would give a better picture of the plant–insect interaction.

The antifeedant activity of the leaf extracts for each species is shown in Figure 4. Activity against both leafrollers is shown by *N. alpina*, *N. dombeyi*, and *N. alessandri*, and these are candidates for chemical examination with the leafroller feeding assay to guide separation. The leaf extracts of *N. obliqua*, *N. obliqua* var. *macrocarpa*, *N. pumilio*, and *N. fusca* showed antifeedant activity against one or another of the leafrollers. Since the leafrollers used in the test are native to New Zealand, it is not unexpected that the leaf extracts of the Chilean species show more frequent activity. Of the New Zealand extracts, only that from *N. fusca* showed activity against a leafroller.

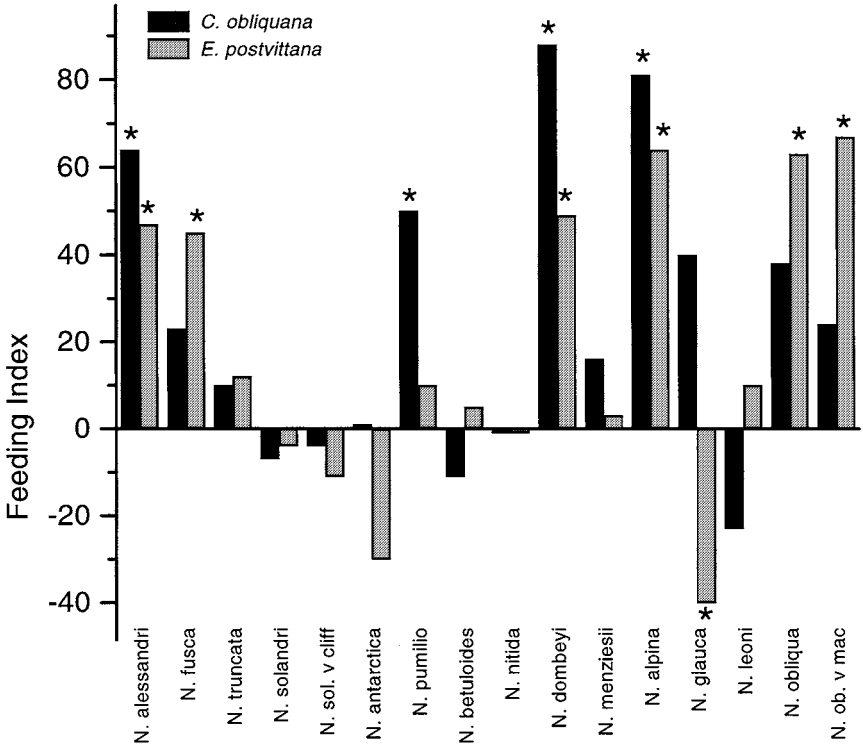

FIG. 4. Antifeedant Activity of *Nothofagus* species against larvae of two leafrollers (*Ctenopsteustis obliquana* and *Epiphyas postvittana*). *Significant difference between treatment and control ($P < 0.05$). Species are ordered according to taxonomic classification (Martin and Dowd, 1993).

The effect of the beech extracts on pea aphid settling is shown in Figure 5a. In nearly all cases, the aphids preferred to settle on bean leaves treated with solvent only, rather than with beech leaf extracts. However, effects ($P < 0.05$) were shown by the leaf extracts of *N. alessandri*, *N. truncata*, *N. solandri*, *N. pumilio*, *N. nitida*, *N. dombeyi*, *N. leoni*, and *N. obliqua*. The effect of the beech extracts on the distribution of produced nymphs is shown in Figure 5b. Most extract-coated leaves had fewer nymphs than control leaves, but the most significant effects ($P < 0.05$) were shown by the leaf extracts of *N. alessandri*, *N. truncata*, *N. antarctica*, *N. pumilio*, *N. dombeyi*, *N. menziesii*, *N. alpina*, *N. leoni*, and *N. obliqua* var. *macrocarpa*. The effect of the extracts on aphid reproduction was indicated by comparison of the number of nymphs produced per adult on each extract-coated leaf with nymphs produced per adult on solvent-treated, control

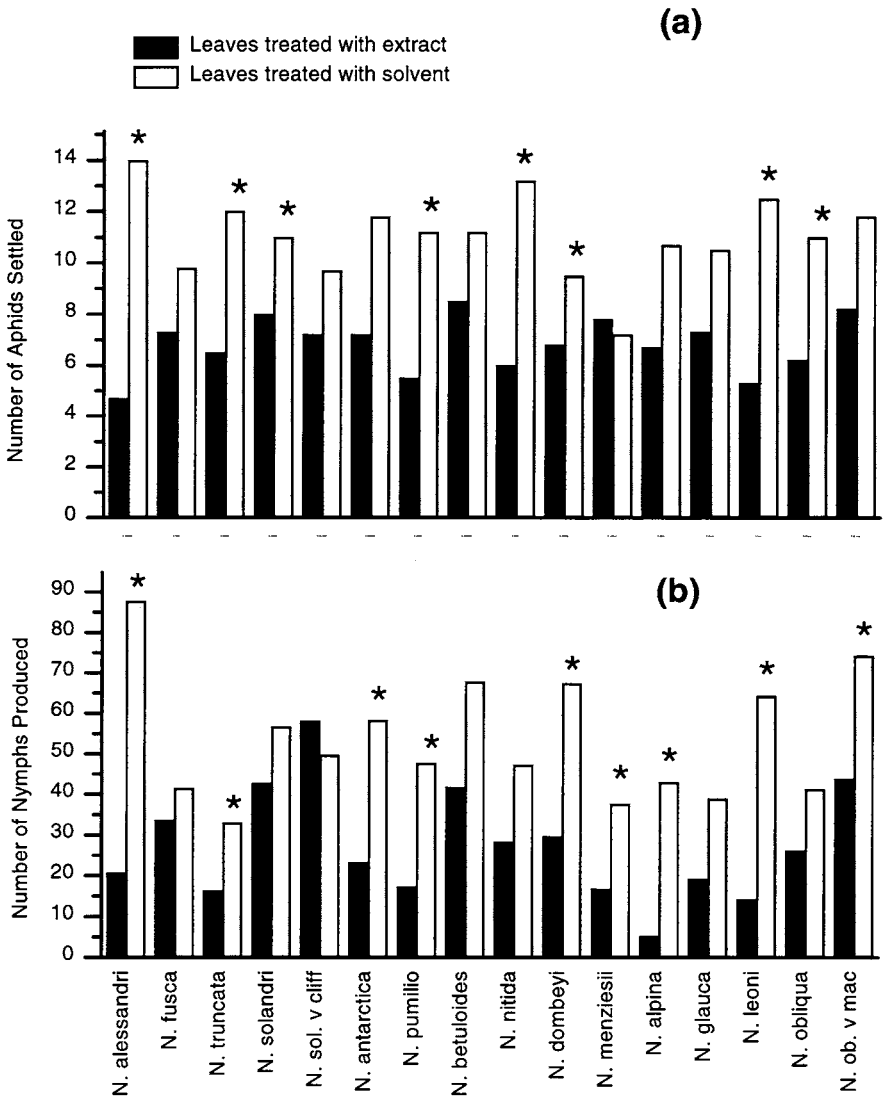

FIG. 5. Effect of *Nothofagus* leaf extracts on adult pea aphid (*Acyrthosiphon pisum*) settling (a) and distribution of produced nymphs (b). *Significant differences between treatment and control ($P < 0.05$). Species are ordered according to taxonomic classification (Martin and Dowd, 1993).

leaves. Significant ($P < 0.05$) reductions in nymph production were shown by extracts of *N. alessandri* (4.4 vs. 6.3), *N. antarctica* (3.2 vs. 4.9), *N. betuliodes* (4.9 vs. 6.1), *N. pumilio* (3.2 vs. 4.3), *N. dombeyi* (4.3 vs. 7.1), *N. menziesii* (2.1 vs. 5.2), *N. alpina* (0.8 vs. 4.0), and *N. leoni* (2.6 vs. 5.1). Extracts affecting both aphid settling and reproduction were those from *N. alessandri*, *N. pumilio*, *N. dombeyi*, and *N. leoni*.

The results of testing the leaf extracts in other laboratory assays are summarized in Table 3. Most extracts gave poor or no activity. There was no juvenile hormone activity, toxicity, or abnormal molting effects against the milkweed bug (*O. faciatus*) when exposed to beech leaf extracts, and the brine shrimp assay showed no invertebrate toxicity. Several extracts showed some apparent nematicidal activity, but the zone inhibitions of *P. solanacarium* growth are below those generally accepted for a positive antibiotic assay (4 mm). In the KB cell test, the extracts from New Zealand *Nothofagus* seemed to give greater toxicity than those from Chilean *Nothofagus*, but most of the test results are below the generally accepted figure for significant toxicity (90%) as compared to standard adriblastine.

The combined results from the arthropod abundance and herbivory survey and from the laboratory assays with the leaf extracts are shown in Table 3. This data matrix highlights *N. alessandri* as a species particularly well defended against herbivory. Plant chemistry may be a major factor contributing to these observations, and, since features of the tree suggest that it is the most primitive species of the genus, a phytochemical study of *Nothofagus* was started with *N. alessandri* to detect possible chemical defense mechanisms and to lay a foundation for a species comparison. *N. pumilio* also showed low leaf damage and activity in the leafroller antifeedant, aphid deterrent, and nematicidal assays and is a candidate for further chemical ecological study.

Solvent partition and flash chromatography of the methanolic leaf extract from *N. alessandri* gave a nonpolar fraction that inhibited feeding by *C. obliquana* larvae. The two major components of this fraction were the flavonoid, galangin (**1**), previously identified in the leaf waxes of *N. antarctica* (Wollenweber et al., 1997), and the stilbene, pinosylvin (**3**), previously found in the heart wood of *N. truncata*, *N. solandri*, and *N. solandri* var. *cliffortoides* (Hillis and Orman, 1962). The flavonoids, 8-methoxygalangin (**3**) and 3-O-methyl-8-methoxygalangin (**4**), were also isolated and identified from NMR and mass spectra.

A TLC comparison of the relevant fractions from leaves of the Chilean and New Zealand *Nothofagus* showed that both pinosylvin and galangin were present only in *N. alessandri* and *N. fusca*, confirming the close taxonomy of these two species in spite of their geographic separation in Chile and New Zealand and explaining the activity shown by *N. fusca* in the antifeeding test with *E. postvittana*. Galangin was tentatively identified in the TLC of *N. antarctica*, *N.*

TABLE 3. INSECT ASSOCIATION AND BIOLOGICAL ACTIVITY OF *Nothofagus* LEAVES

Nothofagus species[a]	Low Insect Abundance[b]	Low Herbivory[c]	Leafroller Antifeeding[d]	Aphid Deterrency[e]	Cytotoxicity[f]	Nematicidal Activity[g]	Antibiotic Activity[h]
alessandri	•	•	•	•	•	•	•
fusca	•		•		•		
solandri							
solandri v cliff.		•			•		
truncata	•	•			•		
antarctica					•		•
pumilio		•	•	•		•	
betuloides						•	
nitida	•	•				•	
dombeyi			•	•			
menziesii	•	•			•		
alpina			•				•
glauca	•	•					•
leoni		•		•		•	
obliqua			•				
obliqua v macro.			•			•	

[a]Species are listed according to taxonomic classification and grouped in subgenera (Martin and Dowd, 1993)—*Fuscospora* (top), *Nothofagus* (middle), and *Lophozonia* (bottom).
[b]Total abundance of associated fauna, <150 individuals.
[c]Leaf feeding damage, <5%.
[d]Significant positive feeding index ($P < 0.05$) for either *C. obliquana* or *E. postvittana*.
[e]Significant reduction in aphid settling and reproduction ($P < 0.05$).
[f]KB cytotoxicity, 30% at 10 μg/liter.
[g]90% mortality at 1 mg/ml.
[h]Bacterial growth zone inhibition, >2 mm.

betuloides, *N. dombeyi*, and *N. pumilio*. *Nothofagus* species have been assigned by Hill and Read (1991) to different subgenera. The distribution of galangin in species of the subgenus *Fuscospora* and *Nothofagus*, and not in the subgenus *Lophozonia*, and of pinosylvin in species of the subgenus *Fuscospora*, and not in the subgenera *Nothofagus* or *Lophozonia*, is consistent with the phylogenetic relationships described for the genus *Nothofagus* based on molecular markers (Martin and Dowd, 1993).

Pinosylvin, galangin, and the two methyl ethers (**3** and **4**) were tested in the two-way choice antifeedant assay but they did not show any significant antifeedant activity at 1% concentration on the feeding squares. A 1 : 1 mixture of galangin and pinosylvin at 1% did show antifeedant activity consistent with the original fraction from which the compounds were isolated. On a dry weight basis, the leaf concentration of galangin and pinosylvin is 0.2% and 0.35%, respectively. If the two compounds are individually active, then at 1% concentration the feeding index of each should have been positive, consistent with the activity of the crude leaf fraction. As it is, it appears that the two compounds act in concert to affect leafroller larval feeding.

In *Pinus*, pinosylvin and other heartwood stilbenes are known for their antifungal activity, have been implicated in the resistance of wood to termite attack, and may appear in sapwood after fungal infection or insect attack (Gorham, 1980 and references therein). In members of the Vitaceae, the stilbenes, resveratrol and its oligomers, have been detected in the leaves after exposure to fungal infection in a typical phytoalexin response (Gorham, 1980). This raises the possibility that *N. alessandri* also has the ability to produce pinosylvin and perhaps galangin in response to insect feeding or fungal infection of the leaves, and the overall effect of these compounds may be magnified by their in situ concentrations about the damaged area of the leaves. While pinosylvin did not show antifeedant activity against leafroller larvae at 1% concentration in the two-way feeding assay, its localized concentration around leaf damage may well be higher and its effect ecologically important. A detailed study of stilbene induction in *Nothofagus* is required to see if this is indeed a major mechanism inhibiting insect feeding or fungal infection and to delineate the compounds and concentrations so produced.

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