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# USE OF A COMPARATIVE APPROACH TO IDENTIFY ALLELOPATHIC POTENTIAL AND RELATIONSHIP BETWEEN ALLELOPATHY BIOASSAYS AND "COMPETITION" EXPERIMENTS FOR TEN GRASSLAND AND PLANT SPECIES

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**Abstract**—Various allelopathy bioassays were used to evaluate the allelopathic potential of 10 grassland forage species against a common test (phytometer) species, *Carduus nutans* L. Aqueous extracts did not influence *C. nutans* germination, although radicle elongation was often severely inhibited. *C. nutans* was strongly affected by shoot, but not root, leachates. Decomposing ground tissue had mixed effects, and often stimulated shoot production of *C. nutans*. Calculation of $R^2$ (coefficient of determination) values between these results, and the results of previous experiments investigating the effects of the same 10 species on *C. nutans* emergence and development in field plots and glasshouse competition experiments frequently revealed strong, statistically significant relationships. Our results therefore provide correlative evidence for the importance of allelopathy in field conditions.

**Key Words**—Allelopathy, bioassay, *Carduus nutans* L., comparative approach, correlative evidence, forage plant, grassland, phytometer.

## INTRODUCTION

Allelopathy has frequently been suggested as an important factor in regulating the structure of plant communities (McPherson et al., 1971; Rice, 1984; Rutherford and Powrie, 1993; Smith and Martin, 1994) and growth rate of plants in

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field conditions (Dias, 1991; Li et al., 1992; Nakahisa et al., 1993). However, many researchers have criticized the notion that allelopathic agents are active or important under field conditions, mainly because of perceived flaws in the methodologies (principally bioassays) used for evaluating allelopathy (Harper, 1977; Keeley, 1988) and because of the failure of one study (Stowe, 1979) to detect a statistically significant relationship between the bioassay results for a range of species and the spatial patterning of the same species in the field.

Comparative approaches to plant ecology often have been utilized successfully for testing hypotheses relating to factors influencing plant growth and structuring plant communities (Gaudet and Keddy, 1988; Grime et al., 1988; Shipley and Peters, 1990; Keddy et al., 1994). Such approaches involve selecting a number of plant species (with each species representing a single independent data point), and investigating whether a statistically significant relationship (usually a correlation) exists between two or more variables measured for the whole range of species tested. Although correlation does not mean causation, predictive comparative approaches can enable prediction of factors important in regulating a wide spectrum of species in a given community (Peters, 1991).

The comparative approach has considerable potential in testing the allelopathy hypothesis. Such a test would involve quantifying the relative allelopathic effect of a range of donor species against a common receiver species (= phytometer; Gaudet and Keddy, 1988) and determining whether these effects were correlated with the relative effects of the same donor species against the receiver species in field (or glasshouse) interference experiments. In previous studies we have reported the relative effects of 10 grassland forage plant species against the seedling emergence, growth, and development of a single test species (the pasture weed *Carduus nutans* L. or nodding thistle) in both field experiments (Wardle et al., 1995) and glasshouse competition experiments (Wardle et al., 1992b). The purposes of the present study were to utilize various conventional bioassay techniques to determine the potential allelopathic effects of the same 10 species on *C. nutans* and to employ the comparative approach to elucidate whether significant relationships could be established between the results of these bioassays and the results of Wardle et al. (1992b, 1995).

## METHODS AND MATERIALS

The donor species used for this study are all used as forage species in New Zealand pastures, and included six grasses [*Dactylis glomerata* L. (cocksfoot), *Phalaris aquatica* L. (phalaris), *Bromus wildenowii* L. (prairie grass), *Lolium perenne* L. (perennial ryegrass), *Festuca arundinacea* Schreb. (tall fescue), and *Holcus lanata* L. (yorkshire fog)] and four legumes [*Medicago sativa* L.

(lucerne), *Trifolium pratense* L. (red clover), *T. subterraneum* L. (subterranean clover), and *T. repens* L. (white clover)]. Seedlings of donor species were raised from commercially available seed sources. Seeds for the receiver species, *C. nutans*, were collected from pastures in the Waikato district of New Zealand [area described in detail by Wardle et al. (1995)], during the summer of 1990–1991. Four bioassays were used to evaluate the effects of each of the 10 species on *C. nutans*: aqueous extract bioassays, leaf leachate bioassays, root leachate bioassays, and decomposing tissue bioassays.

*Tissue Preparation.* To provide tissues for the allelopathy bioassays, polystyrene trays, 50 × 30 × 9 cm deep were filled up to 1 cm depth with a 1 : 1 mixture of soil and pumice, as described by Wardle et al. (1992b). The soil was a Vitric hapludand (Horotiu sandy loam; pH 5.7; organic C 9.1%); the pumice was included to ease the separation of roots. These trays were sown with seeds of each of the 10 species (eight trays per species) at the recommended field sowing rate for each species [rates given by Wardle et al. (1992b)]. Following establishment, the swards were left in glasshouse conditions (temperature range: 10–25°C; day–night light ratio 12 : 12) and amended approximately every two weeks with an appropriate nutrient solution (Smith et al., 1983). After 90 days all the above-ground and below-ground tissue produced by each species was harvested.

*Aqueous Extract Bioassay.* This was conducted according to Wardle et al. (1992a). Subsamples of the shoot and root tissues of each species were air-dried and ground to 0.5 mm using a Wiley mill, mixed with deionized water at a concentration of 5% (w/v), and stirred vigorously at room temperature for 20 min. The mixture was filtered by passing it twice through cheesecloth and then through Whatman No. 1 filter paper. For each tissue, two solutions, 2% and 5% w/v, of tissue were prepared. These concentrations are within the range normally used for bioassays of this type (Stowe, 1979; Hegazy et al., 1990). To assess the allelopathic effect of each solution, 10 replicate 9-cm-diameter Petri dishes were set up and germination tests performed as described by Wardle et al. (1992a). Two pieces of Whatman No. 1 filter paper (9 cm diameter) were added to each dish, to which was added 5 ml of aqueous extract and 20 seeds of *C. nutans*. Controls consisted of 10 Petri dishes containing deionized water in place of the extract. Osmotically adjusted controls were not used (cf. Wardle et al., 1992a) because *C. nutans* is not responsive to osmotic potentials of the extracts. The total number of seeds that germinated for each Petri dish was determined daily for 21 days and seeds were removed upon germination so as to prevent radicles and cotyledons of germinated seeds from interfering with germination of other seeds. Total percent germination for each Petri dish was determined for the whole 21-day period, as well as the speed of germination index, *S*, of Khandakar and Bradbeer (1983) as described by Ahmed and Wardle (1994). This index ranges from 100 (if all seeds germinate on the first day

following set-up) to 0 (if no seeds germinate by the end of the experiment). Assessments of allelopathic effects of these extracts on *C. nutans* radicle elongation were also made, as described by Wardle et al. (1993). Seeds of *C. nutans* were pregerminated on moistened filtered paper, and upon germination were placed in Petri dishes (three seeds per dish) with 5 ml of each of the solutions described above and five replicate dishes per extract. Corresponding controls consisted of deionized water in place of the extract. After 10 days, the length of each radicle was measured.

*Aqueous Leaf Leachate Bioassay.* This bioassay was performed for each donor species as described by Wardle et al. (1993), based on the approach of McPherson et al. (1971) and Stowe (1979). This involved placing 1.5 kg (fresh weight) shoot tissue on a 1-m-diameter sieve (holes = 4 mm) and spraying it with a fine mist of deionized water for approximately 15 min, until 200 ml of leachate was obtained. The effect of each leachate on percent germination, speed of germination, and radicle elongation of *C. nutans* was evaluated exactly as for the aqueous extract bioassay.

*Root Leachate Bioassay.* This bioassay was performed basically as described by Ahmed and Wardle (1994). Six replicate pots (15 cm diameter × 12 cm deep) were prepared for each donor species by filling them with a 1 : 1 mixture of Horotiu silt loam soil and pumice, and sowing them with seeds of that species at the recommended sowing rate (Wardle et al., 1992b). Another six pots, filled with the soil–pumice mix but left unplanted, were set up to serve as controls. All pots were then left in glasshouse conditions as described above, and trimmed every two to three weeks to 5 cm height (to simulate grazing removal in the field; Wardle et al., 1994). After 90 days a further 66 pots were prepared, each filled with the same soil–pumice mix, and planted with 15 *C. nutans* seeds. Each *C. nutans* (receiver) pot was linked with one donor pot (so as to maintain statistical independence). Every seven days for a further 98 days, 650 ml of deionized water was added to the soil surface of each donor pot. Over the following 2 hr, 250 ml of the water that came out of the bottom of each donor pot was collected and added to the soil surface of the corresponding receiver pot. The total number of *C. nutans* seedlings that emerged in each receiver pot was counted approximately every two days for the first three weeks and approximately every five days thereafter until the end of the experiment, so as to determine the total percent seedling emergence and speed of emergence index, $E$ (calculated the same as the speed of germination index, $S$). Thirty-eight days after setting up the receiver pots, the total number of *C. nutans* seedlings was thinned to one per pot, to reduce the potential for intraspecific interference (Wardle et al., 1993). At the end of the experiment, the *C. nutans* plant present in each pot was harvested and the total above-ground and below-ground biomass dry weight, and shoot–root ratio was determined following oven drying (80°C;

24 hr). The entire experiment was performed in glasshouse conditions as described above.

*Decomposing Tissue Bioassay.* This bioassay was performed as described by Wardle et al., (1993). For each donor species, air-dried root and shoot tissue was ground to 0.5 mm using a Wiley mill and mixed with the soil–pumice mixture described above, at each of two concentrations: 0.2% and 0.8% w/w. The concentrations used correspond with the quantities of forage plant tissues present in managed pastures (Wardle et al., 1993). Two types of controls were also used. The first consisted of pumice + soil, without amendments. The second consisted of peat moss mixed with the pumice + soil at concentrations of 0.2% and 0.8%. The rationale behind this second control is that soil organic matter levels are enhanced following addition of plant tissue (resulting in plant responses being attributable to factors other than allelopathy), and the addition of a supposedly inert medium (peat moss) in place of plant tissue is presumed to correct for changes in organic matter without inducing allelopathic effects (Stowe, 1979; Shafer and Garrison, 1986). Six replicate pots (each 15 cm diameter × 12 cm deep) were filled with soil from each tissue type × concentration combination. Fifteen seeds of *C. nutans* were planted in each pot and the total percent seedling emergence and speed of emergence determined as described for the root leachate bioassay. Pots were maintained in glasshouse conditions as described above and amended with an appropriate nutrient solution (Smith et al., 1983). After 36 days the total number of *C. nutans* seedlings in each pot was thinned to one. The experiment was then left for a further 59 days, after which the oven-dry shoot and root weight and shoot–root ratio of each seedling was determined.

*Data Analysis.* Data for each bioassay were calculated as treatment value divided by control value, so as to aid comparisons between bioassays (Wardle et al., 1993; Ahmed and Wardle, 1994). These data were analyzed using analysis of variance testing for donor species effects on *C. nutans*, followed by Tukey's honestly significant difference test at $P = 0.05$.

Coefficients of determination ($R^2$) values were calculated in order to evaluate the relationships between the allelopathy bioassay results and the results of the studies of Wardle et al. (1992b, 1995), who investigated the nature of interference of the 10 forage species on seedling emergence, growth, and development of *C. nutans*. Because of the large number of variables measured for the 10 species (especially in terms of the allelopathy bioassays), if all possible combinations between variables were tested it would be expected that a number of $R^2$ values would emerge as being statistically significant simply due to chance (or type 1 error). To help overcome this problem and to reduce the number of variables in the data set, the data were simplified by using principal components analysis (PCA) to identify principal components that summarized the maximal

variation present across the 10 species for each of the four bioassays (see Wardle and Parkinson, 1990). For example, the six variables relating to the aqueous extracts (germination, speed of germination, and radicle elongation data for each of the 2% and 5% concentrations of extract) were all simplified to one variable, namely the principal component maximizing variation between donor species. Only the first principal component (explaining the greatest variation) was used from each of the four bioassay types, since preliminary analysis revealed that lower-order principal components were unimportant in explaining observed trends. A fifth allelopathy variable was also used, namely the principal component explaining the maximal variation in a previous study aimed at evaluating the effects of residual soil toxicity of each of the 10 donor species against *C. nutans* (Wardle et al., 1991) (hereafter referred to as the residual soil bioassay). $R^2$ analysis was used to elucidate the relationship between each of the five principal components, and the effects of swards of each of the 10 forage species on *C. nutans* seedling emergence, growth rate, mortality, and flowering intensity in the field [data from Wardle et al. (1995)] and on *C. nutans* seedling emergence and growth rate in glasshouse competition experiments [data from Wardle et al. (1992b)].

In a previous study, Wardle et al. (1993) determined the response of the same 10 forage species to the allelopathic potential of *C. nutans* tissues. To determine whether any relationship exists between the allelopathic effect of these 10 species (present study) and their response to allelopathic effects of another species [i.e., whether there exists an allelopathic equivalent of the competitive hierarchy (Panetta and Randall, 1993) of plants], $R^2$ analysis was performed between each of the five principal components described above and the principal components derived from data presented by Wardle et al. (1993). The analyses included principal components summarizing forage plant response to *C. nutans* aqueous extracts, *C. nutans* shoot leachates, and *C. nutans* decomposing tissues.

## RESULTS AND DISCUSSION

*Bioassay Data.* The aqueous extract bioassays showed that total germination and speed of germination of *C. nutans* was generally unresponsive to forage plant tissues at the 2% concentration (Table 1). However, shoot extracts of several species at the 5% concentration inhibited these variables, while root extracts for five of the six grasses significantly enhanced *C. nutans* speed of germination. Radicle elongation had a stronger (and consistently negative) response to shoot and root extracts, supporting other studies that suggest that radicle elongation bioassays are considerably more sensitive than those based on germination (Rice, 1984; Leather and Einhellig, 1986). Since this bioassay is most widely used to evaluate whether water-soluble inhibitors are released

TABLE 1. EFFECT OF AQUEOUS EXTRACTS OF GRASS AND LEGUME TISSUES ON GERMINATION AND RADICLE GROWTH OF *Carduus nutans*[a]

| Tissue type and species | 2% tissue concentration[b] | | | 5% tissue concentration | | |
|---|---|---|---|---|---|---|
| | TG[c] | S | RE | TG | S | RE |
| Shoot tissue | | | | | | |
| *D. glomerata* | 108.6 | 116.1 | 5.3 | 96.0 | 81.2 | 1.3 |
| *P. aquatica* | 110.3 | 112.5 | 4.4 | 23.0 | 19.1 | 0.0 |
| *B. wildenowii* | 104.1 | 103.0 | 5.2 | 94.5 | 86.4 | 4.8 |
| *L. perenne* | 112.8 | 116.9 | 30.0 | 85.5 | 86.4 | 5.2 |
| *F. arundinacea* | 111.5 | 109.6 | 5.7 | 54.3 | 53.6 | 0.0 |
| *H. lanata* | 112.5 | 123.7 | 37.1 | 114.0 | 125.3 | 3.8 |
| *M. sativa* | 113.5 | 95.0 | 21.3 | 55.2 | 39.3 | 5.2 |
| *T. pratense* | 113.2 | 101.5 | 28.4 | 96.0 | 72.3 | 6.7 |
| *T. subterraneum* | 109.0 | 108.2 | 25.5 | 63.7 | 48.4 | 5.8 |
| *T. repens* | 100.0 | 86.3 | 5.2 | 66.7 | 28.2 | 3.6 |
| Tukey's h.s.d.[d] | 15.1 | 20.6 | 19.7 | 22.3 | 22.1 | 6.4 |
| Root tissue | | | | | | |
| *D. glomerata* | 107.1 | 111.9 | 64.5 | 108.2 | 125.6 | 3.7 |
| *P. aquatica* | 115.9 | 142.2 | 62.3 | 112.9 | 133.1 | 10.2 |
| *B. wildenowii* | 108.0 | 119.3 | 45.3 | 110.7 | 131.0 | 13.8 |
| *L. perenne* | 109.6 | 110.1 | 71.7 | 108.6 | 128.8 | 32.9 |
| *F. arundinacea* | 107.9 | 115.8 | 23.9 | 112.8 | 137.8 | 11.6 |
| *H. lanata* | 113.4 | 132.9 | 41.4 | 112.6 | 136.4 | 28.9 |
| *M. sativa* | 104.6 | 95.2 | 11.2 | 83.4 | 63.8 | 5.3 |
| *T. pratense* | 112.1 | 109.8 | 35.6 | 103.7 | 81.7 | 9.5 |
| *T. subterraneum* | 110.1 | 117.0 | 30.1 | 107.0 | 105.5 | 11.3 |
| *T. repens* | 112.8 | 115.5 | 44.5 | 99.0 | 94.7 | 8.5 |
| Tukey's h.s.d. | 14.1 | 19.7 | 29.7 | 16.7 | 22.4 | 13.6 |

[a] All data expressed as (treatment value/control value) × 100.
[b] Weight/volume.
[c] TG, total germination; S, speed of germination index; RE, radicle elongation rate.
[d] Tukey's honestly significant difference at $P = 0.05$.

from dried plant material (Stowe, 1979), it would appear from this bioassay that establishment of seedlings from germinated seeds is likely to be generally negatively affected by the forage species studied.

The aqueous shoot leachates of grasses did not exert strong effects on germination or radicle growth of *C. nutans* (Table 2) except for *L. perenne*, which caused a significant decrease in radicle elongation. However, all four legume species induced a statistically significant stimulation of *C. nutans* speed of germination, and *T. subterraneum* induced a significant decline in *C. nutans* radicle elongation. Since this bioassay is indicative of possible effects of rainfall

TABLE 2. EFFECT OF GRASS AND LEGUME SHOOT LEACHATES ON GERMINATION AND RADICLE GROWTH OF *Carduus nutans*[a]

| Species | TG[b] | S | RE |
|---|---|---|---|
| *D. glomerata* | 106.2 | 105.5 | 83.0 |
| *P. aquatica* | 104.2 | 105.2 | 88.6 |
| *B. wildenowii* | 102.1 | 102.7 | 98.6 |
| *L. perenne* | 94.9 | 98.8 | 64.8 |
| *F. arundinacea* | 105.8 | 106.0 | 82.4 |
| *H. lanata* | 104.3 | 100.1 | 76.7 |
| *M. sativa* | 112.4 | 142.7 | 119.7 |
| *T. pratense* | 103.5 | 123.3 | 94.4 |
| *T. subterraneum* | 99.6 | 135.5 | 67.7 |
| *T. repens* | 101.1 | 129.8 | 110.6 |
| Tukey's h.s.d.[c] | 13.2 | 19.2 | 31.9 |

[a] All data expressed as (treatment value/control value) × 100.
[b] TG, total germination; S, speed of germination index; RE, radicle elongation rate.
[c] Tukey's honestly significant difference at $P = 0.05$.

passing through the leaves of forage plants and reaching target plants situated beneath them (McPherson et al. 1971), it appears unlikely that this mechanism is consistent with suppression of *C. nutans* seedlings by forage species in the field.

There were no significant effects of root leachates on *C. nutans* seedling emergence (Table 3), probably indicative of the high variability of the data. However, both shoot growth and root growth of *C. nutans* were significantly influenced by most of the forage species, with the strongest effects being shown by *P. aquatica*, *L. perenne*, *H. lanata*, and *F. arundinacea*. Nutrient analysis of selected leachates from the donor pots demonstrated that the ammonium, nitrate, and phosphate concentrations did not differ between species (data not shown), so the differential levels of inhibition induced by different species appears to be due to allelopathic, not nutrient depletion effects. For five of the 10 donor species, there was also a significant reduction of *C. nutans* shoot–root ratio, indicating that shoot growth is more susceptible to root leachate toxicity than is root growth. Although this finding is inconsistent with some previous studies (Wardle et al., 1993; Nilsson, 1994), allelopathy involves a wide range of compounds, all of which affect target plants in different ways, so perhaps differences between the results from different studies are not surprising.

The decomposing shoot and root tissues of the donor species did not significantly alter *C. nutans* seedling emergence or rate of emergence (Table 4). However, statistically significant stimulations of *C. nutans* root growth (relative

TABLE 3. EFFECT OF GRASS AND LEGUME ROOT LEACHATES ON EMERGENCE AND GROWTH OF *Carduus nutans*[a]

| Species | TE[b] | SE | S | R | S/R |
|---|---|---|---|---|---|
| *D. glomerata* | 112.4 | 84.2 | 43.2 | 65.9 | 66.9 |
| *P. aquatica* | 112.3 | 89.5 | 41.1 | 65.7 | 64.8 |
| *B. wildenowii* | 114.2 | 85.0 | 57.3 | 76.9 | 75.8 |
| *L. perenne* | 66.2 | 53.6 | 35.9 | 55.8 | 67.7 |
| *F. arundinacea* | 71.2 | 58.0 | 35.7 | 53.9 | 68.2 |
| *H. lanata* | 108.2 | 87.5 | 37.9 | 60.0 | 64.2 |
| *M. sativa* | 99.7 | 83.5 | 82.3 | 95.8 | 86.3 |
| *T. pratense* | 99.9 | 77.4 | 69.9 | 80.1 | 88.2 |
| *T. subterraneum* | 120.3 | 94.0 | 52.6 | 67.3 | 84.7 |
| *T. repens* | 120.7 | 95.6 | 70.5 | 84.6 | 84.3 |
| Tukey's h.s.d.[c] | 66.9 | 49.7 | 17.4 | 27.2 | 34.5 |

[a] All data expressed as (treatment value/control value) × 100.
[b] TE, total emergence of seedlings; SE, speed of emergence index; S, final shoot weight; R, final root weight; S/R, shoot-root ratio.
[c] Tukey's honestly significant difference at $P = 0.05$.

to the unamended controls) were caused by shoot tissues of *D. glomerata* (0.2% concentration), *P. aquatica* (0.2% and 0.8%), *B. wildenowii* (0.2%), *F. arundinacea* (0.2%), and *H. lanata* (0.2 and 0.8%), and by root tissues of *P. aquatica* (0.8%) and *F. arundinacea* (0.8%). However, none of these stimulations were statistically significant when compared with the peat moss-amended control. This suggests that the stimulation of shoot growth may not have been due to allelopathic growth stimulation (Rice, 1986) but rather due to the improvement of soil quality due to organic matter enrichment. Amendment of soil by these tissues could conceivably increase plant growth through enhancing microbial mineralization of plant-available nutrients and enhancing turnover of the soil microbial biomass (Singh et al., 1989; Wardle, 1992).

The bioassay results in combination suggest that different forage plant species may differ in relation to their effects on *C. nutans* establishment. These effects are, however, not consistent across bioassays, probably because aqueous extract and leachate bioassays assess the potential of material to release soluble compounds over a comparatively short period, while decomposition-based bioassays evaluate longer-term processes that affect plant growth and may involve soil microorganisms.

*Correlative Evidence for Allelopathy.* The coefficients of determination ($R^2$) values between the principal component scores for the 10 forage plant species (based on principal components summarizing the data for each of Tables 1–4) and the effects of the same 10 species on *C. nutans* in field plots were often

TABLE 4. EFFECT OF GRASS AND LEGUME TISSUE MIXED WITH SOIL ON EMERGENCE AND GROWTH OF *Carduus nutans*[a]

| Tissue type and species | 0.2% tissue concentration[b] | | | | | 0.8% tissue concentration | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | TE[c] | SE | S | R | S/R | TE | SE | S | R | S/R |
| Shoot tissue | | | | | | | | | | |
| *D. glomerata* | 103 | 111 | 151 | 186 | 81 | 131 | 156 | 184 | 119 | 155 |
| *P. aquatica* | 103 | 114 | 172 | 164 | 105 | 91 | 88 | 250 | 176 | 142 |
| *B. wildenowii* | 114 | 107 | 155 | 165 | 94 | 137 | 146 | 166 | 127 | 131 |
| *L. perenne* | 114 | 132 | 147 | 146 | 101 | 92 | 93 | 232 | 164 | 141 |
| *F. arundinacea* | 117 | 118 | 157 | 184 | 85 | 148 | 143 | 157 | 89 | 176 |
| *H. lanata* | 143 | 150 | 153 | 152 | 102 | 111 | 110 | 213 | 136 | 157 |
| *M. sativa* | 95 | 103 | 130 | 174 | 75 | 77 | 79 | 126 | 184 | 68 |
| *T. pratense* | 69 | 70 | 61 | 55 | 111 | 69 | 65 | 51 | 58 | 88 |
| *T. subterraneum* | 82 | 80 | 92 | 101 | 91 | 95 | 96 | 72 | 102 | 71 |
| *T. repens* | 87 | 85 | 76 | 98 | 78 | 74 | 72 | 77 | 110 | 70 |
| Peat moss | 105 | 113 | 127 | 156 | 81 | 128 | 116 | 128 | 154 | 83 |
| Tukey's h.s.d.[d] | 56 | 62 | 48 | 80 | 39 | 54 | 53 | 93 | 93 | 33 |
| Root tissue | | | | | | | | | | |
| *D. glomerata* | 120 | 125 | 120 | 148 | 81 | 128 | 143 | 145 | 142 | 102 |
| *P. aquatica* | 100 | 103 | 132 | 147 | 90 | 137 | 155 | 180 | 171 | 105 |
| *B. wildenowii* | 160 | 166 | 88 | 92 | 96 | 148 | 150 | 126 | 125 | 101 |
| *L. perenne* | 94 | 99 | 89 | 94 | 94 | 80 | 79 | 86 | 89 | 97 |
| *F. arundinacea* | 117 | 129 | 104 | 103 | 101 | 123 | 114 | 172 | 173 | 99 |
| *H. lanata* | 114 | 127 | 82 | 77 | 106 | 126 | 133 | 91 | 94 | 97 |
| *M. sativa* | 105 | 110 | 108 | 158 | 68 | 72 | 68 | 118 | 148 | 80 |
| *T. pratense* | 90 | 93 | 105 | 116 | 91 | 64 | 67 | 97 | 128 | 76 |
| *T. subterraneum* | 85 | 81 | 132 | 195 | 68 | 59 | 67 | 112 | 150 | 75 |
| *T. repens* | 80 | 77 | 93 | 99 | 94 | 36 | 38 | 105 | 119 | 88 |
| Peat moss | 105 | 113 | 127 | 156 | 81 | 128 | 116 | 128 | 154 | 83 |
| Tukey's h.s.d.[d] | 50 | 66 | 55 | 82 | 30 | 60 | 67 | 62 | 80 | 30 |

[a] All data expressed as (treatment value/control value) × 100; controls used for this calculation are unamended.
[b] Weight/weight.
[c] TE, total emergence of seedlings; SE, speed of emergence index; S, final shoot weight; R, final root weight; S/R, shoot–root ratio.
[d] Tukey's honestly significant difference at $P = 0.05$.

very highly significant (Table 5). This result provides strong support for the likelihood of allelopathy occurring in the field experiment of Wardle et al. (1995), in that over 50% of the variation in *C. nutans* seedling mortality (i.e., those *C. nutans* plants that died before flowering) and the proportion of *C. nutans* plants that flowered as annuals (i.e., those that reached sufficient size in their first year to flower rather than persist as biennials) in field plots could be explained by each of the four bioassays. Further, the decomposing tissue

TABLE 5. COEFFICIENTS OF DETERMINATION ($R^2$) FOR RELATIONSHIPS BETWEEN PRINCIPAL COMPONENTS SUMMARIZING DATA FOR ALLELOPATHY BIOASSAYS OF TEN FORAGE SPECIES AGAINST *Carduus nutans* AND RESPONSE OF *C. nutans* TO SAME 10 SPECIES IN FIELD PLOTS AND GLASSHOUSE COMPETITION EXPERIMENTS

| Experiment | Response variable for *C. nutans* in experiment | Principal component summarizing variation for bioassays of: Aqueous extracts | Aqueous shoot leachates | Aqueous root leachates | Decomposing tissues | Residual soil[a] |
|---|---|---|---|---|---|---|
| Field experiment | mortality in first year[b] | 0.638**[c] | 0.518* | 0.717** | 0.625** | 0.286 |
| (Wardle et al., 1995) | total mortality | 0.644** | 0.517* | 0.701** | 0.659** | 0.292 |
| | proportional annuals[d] | 0.774*** | 0.544* | 0.687** | 0.696** | 0.373 |
| | capitula number per plant | 0.497* | 0.267 | 0.332 | 0.806*** | 0.033 |
| | maximum diameter (rosette)[e] | 0.231 | 0.114 | 0.364 | 0.699** | 0.033 |
| | maximum diameter (flowering) | 0.404* | 0.192 | 0.350 | 0.756** | 0.063 |
| | seedling emergence[f] | 0.483* | 0.273 | 0.284 | 0.416* | 0.117 |
| Glasshouse experiment | shoot weight | 0.508* | 0.275 | 0.598** | 0.676** | 0.510* |
| (Wardle et al., 1992) | root weight | 0.629** | 0.426* | 0.652** | 0.677** | 0.514* |
| | shoot–root ratio | 0.018 | 0.066 | 0.012 | 0.305 | 0.029 |
| | plant diameter | 0.729** | 0.535* | 0.784** | 0.597* | 0.474* |
| | seedling emergence | 0.623*** | 0.452* | 0.452* | 0.238 | 0.578* |

[a] Data from Wardle et al., (1991).
[b] All field experiment data except seedling emergence is based on a labeled cohort of *C. nutans* seedlings in plots previously sown with each of the 10 donor species.
[c] *·**·*** = $R^2$ is significantly different to 0 at $P = 0.05$, 0.01, and 0.001, respectively (for 8 df).
[d] Proportion of labeled plants that flowered as annuals.
[e] Maximum diameter of rosette plant immediately prior to flowering.
[f] Principal component summarizing emergence data collected every 2 to 3 months for $2\frac{1}{2}$ years.

bioassay also explained over 80% of the variation in capitula number produced per *C. nutans* plant across the 10 sward types in the field, suggesting that allelopathy is important in regulating the final size that *C. nutans* plants reach when they flower and, hence, their potential reproductive fitness (or output). Essentially, the same pattern was observed when the results of the four bioassays were compared with the effects of the same 10 species on *C. nutans* in glasshouse competition experiments [data from Wardle et al. (1992b)]—*C. nutans* shoot weight, root weight, plant diameter, and seedling emergence all showed a consistent relationship with each of the four principal components. The principal component summarizing the results of the soil toxicity bioassay study of Wardle et al. (1991) did not, however, show strong relationships with either the field or glasshouse competition experiments.

It should be noted that for two of the bioassays (aqueous extract and decomposing tissue), some donor species stimulated *C. nutans*, while the same species inhibited *C. nutans* in the studies of Wardle et al. (1992b, 1995). However, in competition experiments, both allelopathy and resource competition can occur simultaneously (see Nilsson, 1994), and it could be expected that resource competition by forage species suppresses *C. nutans* growth, so that the overall effect of forage species on *C. nutans* would be considerably more negative than would be predicted by an allelopathy bioassay.

One of the principal criticisms of the allelopathy hypothesis is the supposed failure of bioassay results to correlate with those of field studies or observations [interestingly, the same demands are not usually made for experiments testing for resource competition; see discussion by Williamson, (1990)]. Moreover, the failure of Stowe (1979) to detect a statistical relationship between allelopathy bioassays for seven species and the field patterning of the same seven species is often used as evidence against the allelopathy hypothesis (see, e.g., Keeley, 1988). Our results differed from those of Stowe (1979) in that we were able to detect a statistical relationship; however, this may be due to important differences between the two studies. In our study, the experimental field plots consisted of comparatively pure swards of each of the 10 species, enabling allelopathic compounds of only one species to dominate in each plot. It is therefore possible that this accumulation of allelopathic components can be simulated in a bioassay if an appropriate concentration of tissue is used. Stowe's field site, in contrast, consisted of a diverse range of species, and it is unlikely that any one species would ever reach sufficient dominance for its allelochemicals to dominate subsequent interactions. We propose that effective and consistent allelopathic inhibition of one species by another is more likely to occur in communities of low species richness where the nature of the soil biochemistry is likely to be determined by a dominant plant species. Christensen and Muller (1975) detected a statistically significant relationship between the differential response of 10 herbaceous species to the chaparral shrub *Adenostema fasciculata*

H.&A. in field conditions and their response to the allelopathy bioassays of *A. fasciculata*. Their experiment also supports our hypothesis, since individual *A. fasciculata* shrubs would be much larger than individuals of the other species present and would therefore be more likely to have a dominating effect on the soil biochemical composition. We are aware that correlation does not mean causation, but we believe that our results help to refute one of the main criticisms of allelopathy bioassays—that their results do not correlate well with observed vegetation patterns.

*Comparison of Effects and Responses.* The results of our study can also be used, via a comparative approach, to test hypotheses relating to whether species that are more allelopathic are also those that are less sensitive to allelopathic effects of other species. Values of $R^2$ for allelopathic effects against *C. nutans* vs. allelopathic response to *C. nutans* across the 10 forage species revealed weak and inconsistent relationships (Table 6). In studies of plant competition, it has often been demonstrated that effect and response are inversely correlated (Goldberg and Fleetwood, 1987; Miller and Werner, 1987; Panetta and Randall, 1993) and that a competitive hierarchy therefore exists. No such relationship was detected in the present study, meaning that the two components of allelopathy (effect and response) are unrelated and that there is little evidence for an allelopathic hierarchy. If such a hierarchy does not exist, it could be expected that wherever allelopathy is important in field conditions, species spatial patterning would be much less predictable than in circumstances where competition is important.

*Implications for Biological Control.* One of the most promising applications

TABLE 6. COEFFICIENTS OF DETERMINATION ($R^2$) FOR RELATIONSHIPS BETWEEN PRINCIPAL COMPONENTS SUMMARIZING BIOASSAY DATA FOR ALLELOPATHIC EFFECTS OF 10 FORAGE SPECIES AGAINST *Carduus nutans* AND RESPONSE OF SAME 10 SPECIES TO ALLELOPATHIC EFFECTS OF *C. nutans*[a]

| Principal component summarizing response of forage species to *C. nutans* for bioassay of | Principal component summarizing effect of forage species on *C. nutans* for bioassays of | | | | |
|---|---|---|---|---|---|
| | Aqueous extracts | Aqueous shoot leachates | Aqueous root leachates | Decomposing tissues | Residual soil[b] |
| Aqueous extracts | 0.346 | 0.116 | 0.471*[c] | 0.494* | 0.241 |
| Aqueous shoot leachates | 0.436* | 0.169 | 0.139 | 0.476* | 0.197 |
| Decomposing tissues | 0.246 | 0.319 | 0.038 | 0.006 | 0.017 |

[a] Response data from Wardle et al. (1993).
[b] Data from Wardle et al. (1991).
[c] *·**·*** = $R^2$ is significantly different to 0 at $P = 0.05$, 0.01, and 0.001, respectively (for 8 *df*).

of allelopathy is for biological control of weeds (Altieri and Doll, 1978; Lovett, 1990). Our phytometer species, *C. nutans*, is a serious weed in many temperate regions, including New Zealand (Popay and Medd, 1990), where it aggressively competes with forage plants and reduces potential production available for livestock (Thompson et al., 1987). In our previous field study (Wardle et al., 1995) we demonstrated that interference from forage species was extremely important in regulating *C. nutans* population density, growth rate, and reproductive output of individual plants. The present study has provided evidence, through the use of a comparative approach, that this inhibition has an allelopathic basis and that forage species and cultivars with strong allelopathic effects may have a considerable and largely unrealized potential in the management of problematic weed species such as *C. nutans*.

## REFERENCES

AHMED, M., and WARDLE, D. A. 1994. Allelopathic potential of vegetative and flowering ragwort (*Senecio jacobaea* L.) plants against associated pasture species. *Plant Soil* 164:61–68.

ALTIERI, M. A., and DOLL, J. D. 1978. The potential of allelopathy as a tool for weed management in field crops. *PANS* 24:495–507.

CHRISTENSEN, N. L., and MULLER, C. H. 1975. Effects of fire on factors controlling plant growth in *Adenostema* chaparral. *Ecol. Monogr.* 45:29–55.

DIAS, L. S. 1991. Allelopathic activity of decomposing straw of wheat and oat and associated soil on some crop species. *Soil Till. Res.* 21:113–120.

GAUDET, C. L., and KEDDY, P. A. 1988. A comparative approach to predicting competitive ability from plant traits. *Nature* 334:242–243.

GOLDBERG, D. E., and FLEETWOOD, L. 1987. Competitive effect and response in four annual plants. *J. Ecol.* 75:1131–1143.

GRIME, J. P., HODGSON, J. G., and HUNT, R. 1988. Comparative Plant Ecology: A Functional Approach to Common British Species. Unwin Hyman, London.

HARPER, J. L. 1977. Population Biology of Plants. Academic Press, London.

HEGAZY, A. K., MANSOUR, K. S., and ABDEL-HADY, N. F. 1990. Allelopathic and autotoxic effects of *Anastatica hierochuntica* L. *J. Chem. Ecol.* 16:2183–2193.

KEDDY, P. A., TWOLAND-STRUTT, L., and WISHEU, I. C. 1994. Competitive effect and response ranking in 20 wetland plants: Are they consistent across three environments? *J. Ecol.* 82:635–643.

KEELEY, J. E. 1988. Allelopathy. *Ecology* 69:262–263.

KHANDAKAR, A. L., and BRADBEER, J. W. 1983. Jute Seed Quality. Bangladesh Agricultural Research Council, Dhaka.

LEATHER, G. R., and EINHELLIG, F. A. 1986. Bioassays in the study of allelopathy, pp. 133–145, *in* A. R. Putnam and C.-S. Tang (eds.). The Science of Allelopathy. John Wiley & Sons, New York.

LI, H. H., NISHIMURA, H., HASEGAWA, K., and MIZUTANI, J. 1992. Allelopathy of *Sasa cernua*. *J. Chem. Ecol.* 18:1785–1796.

LOVETT, J. V. 1990. Chemicals in plant protection: is there a natural alternative? pp. 57–65, *in* C. Bassett, L. J. Whitehouse, and J. A. Zabkiewicz (eds.). Alternatives to the Chemical Control of Weeds. Ministry of Forestry, Rotorua.

McPherson, J. K., Chou, C.-H., and Muller, C. H. 1971. Allelopathic constituents of the chaparral shrub *Adenostema fasciculata*. *Phytochemistry* 10:2925–2933.

Miller, T. E., and Werner, P. A. 1987. Competitive effects and responses between plant species in a first-year old-field community. *Ecology* 68:1201–1210.

Nakahisa, K., Tsuzuki, E., and Mitsumizo, T. 1993. Study on the allelopathy of alfalfa (*Medicago sativa* L.) I. Observation of allelopathy and survey for substances inducing growth inhibition. *Jpn. J. Crop Sci.* 62:294–299.

Nilsson, M.-C. 1994. Separation of allelopathy and resource competition by the boreal dwarf shrub *Empetrum hermaphroditum* Hagerup. *Oecologia* 98:1–7.

Panetta, F. D., and Randall, R. P. 1993. *Emex australis* and the competitive hierarchy of a grazed annual pasture. *J. Appl. Ecol.* 30:373–379.

Peters, R. H. 1991. A Critique of Ecology. Cambridge University Press, Cambridge.

Popay, A. I., and Medd, R. W. 1990. The biology of Australian weeds. 21. *Carduus nutans* L. ssp. *nutans*. *Plant Prot. Q.* 5:3–13.

Rice, E. L. 1984. Allelopathy, 2nd ed. Academic Press, Orlando.

Rice, E. L. 1986. Allelopathic growth stimulation, pp. 23–41, *in* A. R. Putnam and C.-S. Tang (eds.). The Science of Allelopathy. John Wiley & Sons, New York.

Rutherford, M. C., and Powrie, L. W. 1993. Allelochemic control of biomass allocation in interacting shrub species. *J. Chem. Ecol.* 19:893–906.

Shafer, W. E., and Garrison, S. A. 1986. Allelopathic effects of soil-incorporated asparagus roots on lettuce, tomato and asparagus seedling emergence. *Hortscience* 21:82–84.

Shipley, B., and Peters, R. H. 1990. A test of the Tilman model of plant strategies: Relative growth rate and biomass partitioning. *Am. Nat.* 136:139–153.

Singh, J. S., Raghubanshi, A. S., Singh, R. S., and Srivastava, S. C. 1989. Microbial biomass acts as a source and sink of nutrients in dry tropical forest and savanna. *Nature* 338:499–500.

Smith, A. E., and Martin, L. D. 1994. Allelopathic characteristics of three cool-season grass species in the forage ecosystem. *Agron. J.* 86:243–246.

Smith, G. S., Johnson, C. M., and Cornforth, I. S. 1983. Comparison of nutrient solutions for growth of plants in sand culture. *New Phytol.* 94:537–548.

Stowe, L. G. 1979. Allelopathy and its influence on the distribution of plants in an Illinois old field. *J. Ecol.* 67:1065–1085.

Thompson, A., Saunders, A. E., and Martin, P. 1987. The effect of nodding thistle (*Carduus nutans*) on pasture production. Proceedings of the 40th New Zealand Weed and Pest Control Conference, pp. 222–225.

Wardle, D. A. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biol. Rev.* 67:321–358.

Wardle, D. A., and Parkinson, D. 1990. Interactions between microclimatic variables and the soil microbial biomass. *Biol. Fertil. Soils* 9:272–280.

Wardle, D. A., Nicholson, K. S., and Ahmed, M. 1991. Residual allelopathic effects of pasture grasses and legumes against nodding thistle seedling emergence and growth. Proceedings of the 44th New Zealand Weed and Pest Control Conference, pp. 284–287.

Wardle, D. A., Nicholson, K. S., and Ahmed, M. 1992a. Comparison of osmotic and allelopathic effects of grass leaf extracts on grass seed germination and radicle elongation. *Plant Soil* 140:315–319.

Wardle, D. A., Nicholson, K. S., and Rahman, A. 1992b. Influence of pasture grass and legume swards on seedling emergence and growth of *Carduus nutans* L. and *Cirsium vulgare* L. *Weed Res.* 32:119–128.

Wardle, D. A., Nicholson, K. S., and Rahman, A. 1993. Influence of plant age on the allelopathic potential of nodding thistle (*Carduus nutans* L.) against pasture grasses and legumes. *Weed Res.* 33:69–78.

WARDLE, D. A., NICHOLSON, K. S., AHMED, M. and RAHMAN, A. 1994. Interference effects of the invasive plant *Carduus nutans* L. against the nitrogen fixation ability of *Trifolium repens* L. *Plant Soil* 163:287–297.

WARDLE, D. A., NICHOLSON, K. S., AHMED, M., and RAHMAN, A. 1995. Influence of forage plants on seedling emergence, growth and development of *Carduus nutans* L. *J. Appl. Ecol.* 32:225–233.

WILLIAMSON, G. B. 1990. Allelopathy, Koch's postulates and the neck riddle, pp. 142–162, *in* J. B. Grace and D. Tilman (eds.). Perspectives on Plant Competition. Academic Press, San Diego.