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Interference effects of the invasive plant *Carduus nutans* L. against the nitrogen fixation ability of *Trifolium repens* L.

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Abstract

Carduus nutans L. is an invasive pasture/grassland species which may undergo rapid population growth through positive feedback. Plants of *C. nutans* produce a vegetative rosette, and after several months produce stems containing flower-heads, during which time the rosette leaves die and decompose. We investigated the influence of *C. nutans* on the nitrogen-fixation ability of *Trifolium repens* L. in three experiments. The first experiment was set up in a "mixture" design, and demonstrated that seedlings of *T. repens* were more susceptible to competition with other *T. repens* seedlings than to *C. nutans* seedlings. Nodule numbers and acetylene reduction per unit root, and acetylene reduction per unit nodules were adversely affected by increasing *T. repens*, but not *C. nutans* densities. The second experiment was of an additive design, with separate partitions to isolate above-ground and below-ground interference. Flowering *C. nutans* plants strongly inhibited *T. repens* root growth, nodulation and acetylene reduction, but usually only when shoot interference was permitted. This appears to be due to decomposition of rosette leaves, which was maximal at this stage. The third experiment involved monitoring effects of tagged *C. nutans* individuals against *T. repens* in the field. This experiment showed that acetylene reduction was severely influenced by flowering *C. nutans* (when rosette leaves were decomposing), even when only mild reduction of *T. repens* growth was observed, and these effects persisted for some months after the *C. nutans* plants had died. The results of these experiments in combination suggest that decomposing rosette leaves have a strong potential to inhibit *T. repens* nitrogen fixation. It appears that allelopathy is involved, since alternative explanations (e.g. root competition by *C. nutans*; effects of *C. nutans* on soil moisture, microbial nutrient immobilisation and light availability; facilitation of herbivores by *C. nutans*) can be effectively discounted. Although invasive species are often assumed to be associated with soil nitrogen build-up, we believe that some invasive species such as *C. nutans* have the potential to induce long-term decline of soil nitrogen input.

Introduction

Most plant species are capable of influencing the quality of their environment, and this is likely to be important in determining subsequent plant species composition (Clements, 1936; Miles, 1985; Roberts, 1987). Plants may exert substantial effects on nutrient cycling, and it is increasingly becoming recognised that these effects may be as important as the effects of abiotic factors in controlling soil fertility (Hobbie, 1992). Some plant species modify soil fertility so as to make conditions more suitable for other species, facilitating succession (Finegan, 1984; Pickett *et al.*, 1987), while others adjust their environment to their own advantage, creating a positive feedback situation (Wilson and Agnew, 1992). Nitrogen fixation by both symbiotic and free-living bacteria is highly sensitive to interference effects (including allelopathy) from certain plant species (Rice, 1992; Weston and Putnam, 1985), resulting in a situation where plant species tolerant of low nitrogen status are favoured (Wilson and Agnew, 1992).

Carduus nutans L. (nodding or musk thistle) is an invasive annual or biennial plant species in many temperate grassland systems throughout the world. In New Zealand it can be abundant in perennial pastures dominated by *Lolium perenne* L. (perennial ryegrass) and *Trifolium repens* L. (white clover). It requires gaps of around 6 cm diameter in pasture to establish (Panetta and Wardle, 1992) and develops poorly in vigorously growing pasture (Wardle *et al.*, 1992, 1994).

Although *C. nutans* is sometimes associated with fertile soils (Doing *et al.*, 1969) we have observed it to increase in situations of declining fertility, mainly because the interference potential of associated species declines. *C. nutans* produces a vegetative rosette of up to 80 cm diameter in conditions of low pasture interference, and at flowering the rosette leaves die and rapidly decompose, releasing allelopathic substances which may be highly effective at suppressing legumes (Wardle *et al.*, 1993a). Suppression of pasture by *C. nutans* often results in the creation of gaps in which *C. nutans* will preferentially re-establish (Wardle *et al.*, 1993b).

Individual *C. nutans* plants may reach a large size relative to associated plant species, and therefore is an ideal model species for investigating the potential effects of invasive, rapidly growing short-lived plants on below-ground processes including nutrient cycling. The ability of *C. nutans* to severely suppress *T. repens* suggests that the nitrogen fixation process may be especially susceptible to the inhibitory effects of this weed. The purpose of the present study was to utilise experimental approaches for studying plant "competition", to investigate the potential inhibitory effects of *C. nutans* L. against the potential nitrogen-fixing ability of *T. repens*.

Materials and methods

Three experiments were set up to investigate the potential interference effects of *C. nutans* against the nitrogen-fixing potential of *T. repens*, and these will be referred to throughout the paper as the "mixture experiment", "additive experiment" and "field experiment".

Mixture experiment

This experiment was conducted to determine whether high densities of *C. nutans* seedlings could exert strong effects against *T. repens* nitrogen fixation potential. The experimental design is based upon the "mixture" approach advocated by Firbank and Watkinson (1985) and Law and Watkinson (1987), where the total density as well as the relative proportions of the component species are varied, so as to overcome the disadvantages of the traditional replacement-series approach (Connolly, 1988; Snaydon, 1991).

The experiment was set up on 15–17 May 1991, in polystyrene trays 50 × 30 × 9 cm deep, filled to the surface with a 1:1 mixture of pumice and soil (Wardle *et al.*, 1992). The soil used was a Vitric hapludand (Horotiu sandy loam; pH 5.7; organic C 9.1%); the pumice was included to enable separation of growth substrate from the roots later in the experiment. Pregerminated *C. nutans* and *T. repens* seeds were planted on these trays to yield 25 treatments, i.e. all the possible two-way combinations of *C. nutans* (0, 67, 133, 266 and 532 plants/m^2) × *T. repens* (0, 67, 133, 266 and 532 plants/m^2). The density of 266 seedlings/m^2 of *T. repens* most closely approximates the recommended field sowing rate of this species. The experiment was set up in a randomised block design with four replicates per combination.

Following seedling emergence these trays were left to establish in glasshouse conditions, and amended every two weeks with appropriate levels of nitrogen-free nutrient solution (Smith *et al.*, 1983; Wardle *et al.*, 1992). Glasshouse temperatures varied between 10°C and 25°C, and the day:night light ratio was around 12h:12h throughout the experimental period. After 131 days (23–25 September 1991) each tray was harvested. The following assessments were made for each tray:

(i) Total number of seedlings surviving for each species.

(ii) Total shoot dry weight of each species.

(iii) One quarter of the soil in the tray was removed and all roots present were carefully hand-sorted into *T. repens* or *C. nutans*. The total number of nodules on a preweighed subsample (1–2g wet weight) of *T. repens* roots was counted and the wet and dry weight (80°C, 24h) of the total roots of each species was determined.

(iv) The acetylene reduction bioassay was used to assess N-fixation across the different treatments. Although there are conflicting views on the reliability of the acetylene reduction technique (cf. Minchin *et al.*, 1994; Vessey, 1994), in the present study this method was used only to provide relative estimates of nitrogenase activity, and to complement the nodulation and plant biomass data. Broadly, the approach of Hoglund and Brock (1978) was used. In the remaining three-quarters of the tray not used in (iii) above, eight soil cores (each 2.5 cm diameter × 9 cm depth) were removed and placed into a 1 L sealed glass jar. Cores were all maintained intact and were not detopped, so as to minimise soil disturbance effects (Minchin *et al.*, 1986). For each jar, 50 mL of headspace air was removed and replaced by 50 mL analytical-grade acetylene (New Zealand Industrial Gases). Although nitrogenase activity is known to be reduced in the presence of acetylene (Minchin *et al.*, 1983), in our study there was no evidence for plant density-dependent effects on acetylene-induced decline, and we therefore assumed that any error would be consistent among treatments. After 4 h the ethylene concentration of the headspace was determined on a Tracor S70 Gas Chromatograph with a flame-ionisation detector and a Ni column (1.5 m × 2.0 mm inside diameter) operated at 60°C and packed with Poropaq T (Tough and Crush, 1979). The total headspace volume was then determined for each jar by water displacement. Blanks were used for assessing background ethylene levels.

Additive experiment

To determine the potential effects of individual older *C. nutans* plants on nitrogen fixation, an additive design was used. Additive approaches have the advantage in enabling assessment of interference effects of plants of vastly different sizes on each other (Gaudet and Keddy, 1988; Snaydon, 1991). Trays were filled with soil-pumice mix as described earlier. On 9–10 May 1991, one pre-germinated thistle seed was planted in each tray, 8 cm from one end. Pre-germinated *T. repens* seeds were then placed in four concentric arcs, at 6, 12, 20 and 28 cm from the thistle seed. Within each arc, four *T. repens* seedlings were established, each 5 cm from each other. Four possible interference treatments were then established to assess the relative importance of shoot and root interference (Donald, 1958; Groves and Williams 1975; Wilson, 1988) i.e.

(i) Root interference only. An aerial partition (20 cm high) was placed between the *C. nutans* seedling and the innermost arc of *T. repens* seedlings; the bottom of the partition was touching the soil surface. This partition was covered in reflective aluminium foil to minimise shading effects. Only below-ground interactions are possible.
(ii) Shoot interference only. A below-ground partition was placed as described in (i) above, with its uppermost edge at the soil surface. Only above-ground interactions are possible.
(iii) Shoot and root interference. No partitions were present.
(iv) No interference. Partitions were established both above-ground and below-ground.

Twenty trays were set up for each treatment. These trays were maintained in glasshouse conditions as described earlier, and clover vegetation height was regularly trimmed to a 4–6 cm height to simulate grazing removal (Wardle *et al.*, 1992). Trays were removed and destructively harvested for each treatment at four separate sampling dates:

(i) 2 September 1991 (116 days), when thistles were small rosettes.
(ii) 11 December 1991 (216 days), when thistles were full-sized rosettes.
(iii) 5 February 1992 (272 days), when those thistles which behaved as annuals were in full flower.
(iv) 3 December 1992 (574 days), when those thistles which behaved as biennials were in full flower.

Four replicates per treatment were harvested for each of the first two (non-flowering) sampling dates. However, for the third and fourth sampling dates only 2 and 3 replicates respectively were harvested for each treatment. This lesser replication is because *C. nutans* flowering patterns within cohorts is extremely poorly synchronised (Wardle *et al.*, 1994), and only a small proportion of thistle seedlings were flowering at any one time. This level of replication was deemed to be sufficient to detect important trends. For each tray harvested the following assessments were made:

(i) Oven-dry weight (above-ground and below-ground) for the thistles present.
(ii) For each of the four arcs, four 5.0 cm diameter cores were removed. For two cores the soil was removed from the clover roots, and the nodule number and root biomass were determined as discussed earlier. It was not possible to identify individual clover plants, since they were extensively stolonated by the second sampling date, and all assessments were thus interpreted on an areal basis.
(iii) For the two remaining cores in (ii) above, acetylene reduction was assessed as described for the mixture experiment.

Field experiment

To determine the applicability of the results of the additive experiment to a field situation, a study was conducted to assess potential inhibitory effects of *C. nutans*. An area of grazed perennial *L. perenne*/*T. repens* pasture of approximately 0.2 ha was selected near Ohaupo, New Zealand. This site has been described in detail elsewhere (Panetta and Wardle, 1992; Wardle *et al.*, 1994). The area was grazed by cattle on a 40-day rotation with a mean stocking rate of 2.2 cows/ha throughout the experiment. Within this area, thistles which emerged during the autumn (April-May) of 1992 were initially labelled with 10 cm plastic pegs and later with 40 cm wooden pegs, and where necessary thinned so as to eliminate possible intraspecific interference. Assessments were made when thistles were at the large rosette stage (24 September 1992), full-flowering stage (9 December 1992), senescent phase (17 February 1993) and dead or absent phase (13 April, 1 July and 21 September 1993). Eight thistles were used for each assessment.

At the 24 September 1992 sampling, three zones were identified for each thistle, viz. the area under the rosette (weed or "W" zone), a 10 cm wide ring around the outside of the weed zone (ring or "R" zone), and a corresponding 0.25 m × 0.25 m square of pasture about 2–3 m from the thistle, intended to serve as a control (pasture or "P" zone). At this sampling, the diameter of all labelled thistles was recorded, and after the rosette leaves of the thistles died (i.e. the subsequent samplings), the "W" zone applied to the area that the rosette previously occupied while as a large rosette.

Two weeks prior to each sampling date the pasture vegetation in the W, R and P zones for each thistle was trimmed to about 1 cm height. At this stage livestock was excluded until sampling. On the sampling date the pasture was again clipped to 1 cm height and all vegetation produced over the two week period in each zone was collected. This vegetative material was separated into four categories by hand for each sample (or a pre-weighed subsample), viz. *L. perenne*, other grasses (mainly *Poa annua* L.), *T. repens* and other dicotyledonous species. Plant material was then oven-dried (80°C, 24 h) to enable assessment of above-ground primary productivity for each of the four categories on an areal basis. Damage due to herbivores during this two-week period was presumed to be negligible as herbivore densities in these systems (other than livestock) are characteristically very low.

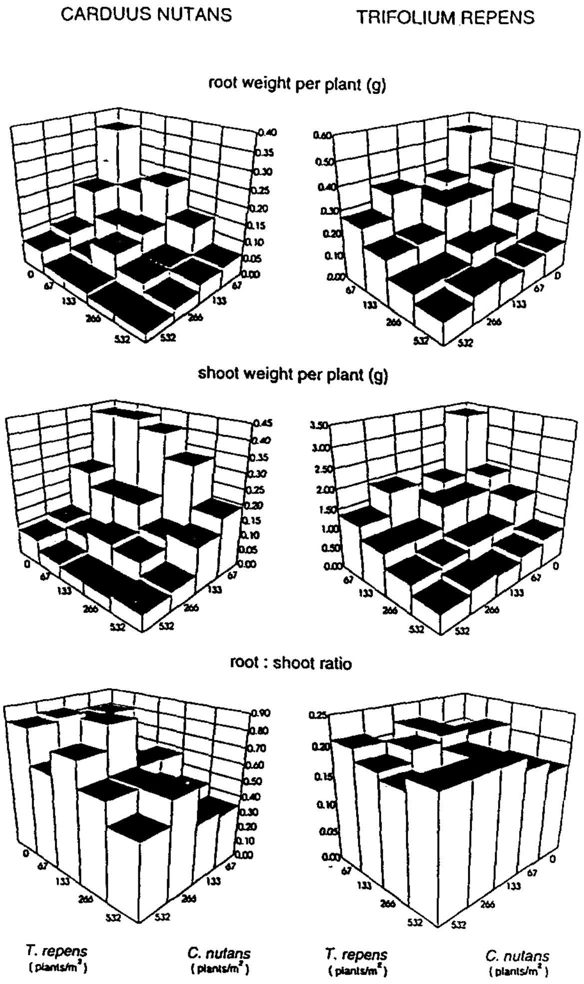

Fig. 1. Root and shoot growth data of *Carduus nutans* and *Trifolium repens* in a mixture experiment. Statistical analyses are presented in Table 1.

At each sampling date, twelve soil cores (each 2.5 cm diameter × 5 cm depth) were collected from the W, R and P zones from each chosen thistle. Six cores were used for assessing acetylene reduction as described for the mixture experiment. Because of the stolonating nature of *T. repens* and the tendency to produce relatively short roots particularly in no-tillage, high rainfall areas, it would be reasonable to expect the vast proportion of roots and nodules to occur in the 0–5 cm layer (cf. Dunlop and Hart, 1987). The other six cores were passed through a 4 mm sieve and used for assessing gravimetric soil moisture (20 g wet weight; 80°C, 24 h), soil basal respiration (BR) and substrate-induced respiration (SIR) measurements. These were determined exactly as described by Wardle *et al.* (1993c), based on approaches by Anderson and Domsch (1978) and West and Sparling (1986). SIR is presumed to be a relative measure of the glucose-responsive ("physiologically active") microbial biomass (Wardle and Parkinson, 1990).

Table 1. Equations for the response of *Carduus nutans* and *Trifolium repens* seedling growth, *T. repens* nodulation and acetylene reduction, to initial planting density of both species (mixture experiment, see Figs. 1–3)

Response variable (Y)	Regression equation	R^2	Significance of coefficients CN	Significance of coefficients TR
C. nutans root dry weight (g)[a]	$Y=-0.90-0.55CN-0.23TR$	0.66	$***$	$***$
T. repens root dry weight (g)[a]	$Y=-0.55-0.084CN-0.39TR$	0.71	$*$	$***$
C. nutans shoot dry weight (g)[a]	$Y=-0.22-0.65CN-0.13TR$	0.81	$***$	$**$
T. repens root dry weight (g)[a]	$Y=1.03-0.082CN-0.36TR$	0.70	$*$	$***$
C. nutans root : shoot ratio	$Y=0.51+0.056CN-0.043TR$	0.19	$**$	$*$
T. repens root : shoot ratio	no significant terms		NS	NS
Competitive balance (roots)	$Y=0.60-0.41TR$	0.31	NS	$***$
Competitive balance (shoots)	$Y=0.89-0.28TR$	0.19	NS	$***$
Nodule number per *T. repens* plant[a]	$Y=6.41-0.39TR$	0.58	NS	$***$
Ethylene production (μmol/plant/h)[a]	$Y=2.53-0.54TR$	0.84	NS	$***$
Ethylene production (μmol/g root/h)[a]	$Y=3.29-0.16TR$	0.18	NS	$***$
Ethylene production (μmol/thousand nodules/h)[a]	$Y=-3.68-0.16TR$	0.15	NS	$**$

Data analysed includes only treatments where initial planting densities of both species were > 0; terms are included only if they significantly enhance R^2.

CN, TR = initial planting density of *C. nutans* and *T. repens*, following $\log_2$ (X/67)-1 transformation.

NS, $*$, $**$, $***$ indicate $p > 0.05$, and $p < 0.05$, 0.01 or 0.001 respectively.

[a] Data analysed following $\log_e$ X-transformation of Y-variate.

Table 2. Trifolium repens nodule density in soil (numbers per dm^2) at different distances from *Carduus nutans* plants, in response to above-ground and below-ground interference (additive experiment)

Developmental stage of *C. nutans*	Nature of interference from *C. nutans*	Distance from *C. nutans* 6 cm	12 cm	20 cm	28 cm
Rosette (vegetative) (11 December 1991)	root + shoot	1326b	1384a	1168a	1186a
	shoot	1650ab	1165bc	1318a	1346a
	root	1315b	1382ab	1357a	1145a
	none	1714a	1150c	1349a	1234a
Flowering (annual plants) (5 February 1992)	root + shoot	821b	900a	885a	905a
	shoot	839b	938a	867a	977a
	root	847b	1020a	885a	882a
	none	972a	1020a	887a	870a
Flowering (biennial plants) (3 December 1992)	root + shoot	275c	431b	477b	612a
	shoot	235c	306c	543ab	558a
	root	405b	505ab	594a	609a
	none	518a	527a	576a	609a

Within each developmental stage × distance combination, numbers followed by different letters are significantly different at $p = 0.05$ (least significant difference test).

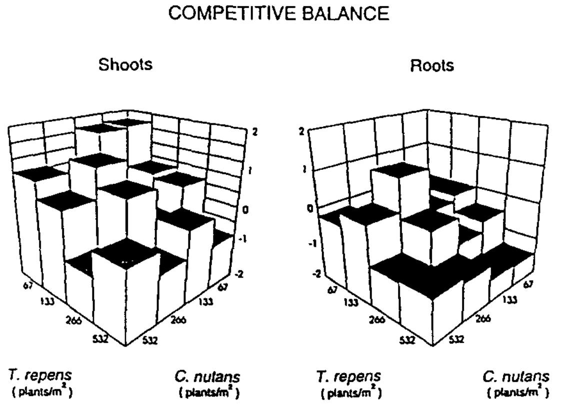

Fig. 2. Competitive balance values between *Carduus nutans* and *Trifolium repens* in a mixutre experiment. These values have been calculated as according to Wilson (1988) and are increasingly positive or negative as *C. nutans* or *T. repens* has the competitive advantage respectively. Statistical analyses are presented in Table 1.

Results and discussion

Mixture experiment

In the mixture experiment, both *C. nutans* and *T. repens* seedlings were more susceptible to intraspecific than interspecific interference, although both types of interference inhibited shoot and root growth for both species (Table 1, Fig. 1). This is reflective of the importance of self-thinning in plant populations (Norberg, 1988). The root: shoot ratio of *T. repens* was independent of initial density of either species, while that of *C. nutans* was enhanced by increasing density of its own species, probably because the prostrate nature of the aboveground portion intensifies above-ground competition for space. Relative competitive ability of *T. repens* and *C. nutans* was assessed using the competitive balance index (Wilson, 1988); in our study an increasing positive or negative value of this index is indicative of *C. nutans* or *T. repens* respectively having the competitive advantage. The competitive balance favoured *C. nutans* at low planting densities of *T. repens* seedlings, but this index significantly declined with increased planting density of *T. repens*. (Fig. 1, Table 2). Relative competitive balances determined using shoot and root data both showed similar trends, particularly at higher *T. repens* densities.

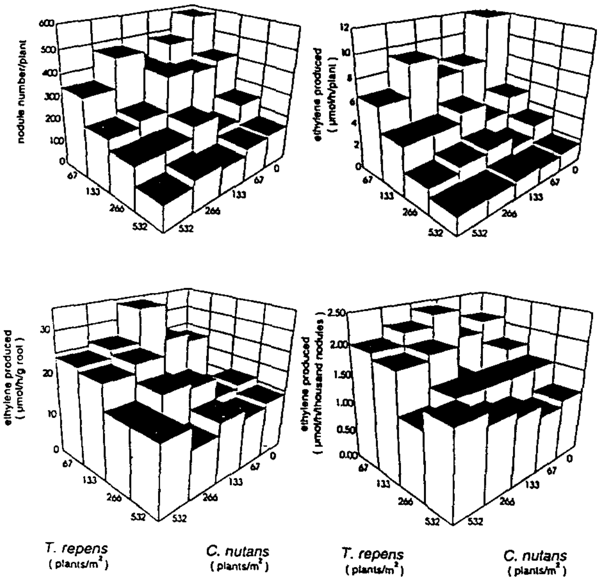

Fig. 3. Nodulation of *Trifolium repens*, and acetylene reduction (ethylene production) in a mixture experiment. Statistical analyses are presented in Table 1.

The strong negative effects that *T. repens* seedlings exerted on each other were reflected in the nodulation data. Nodule number per plant was adversely influenced by increasing *T. repens* density (Fig. 3, Table 1) but this appeared to be entirely related to the reduction of root biomass, since the nodule number per unit weight of root was independent of plant density (data not presented). Acetylene reduction per plant also declined with increasing *T. repens* density, but in addition there was a statistically significant effect of *T. repens* density on acetylene reduction per g weight roots and per 1000 nodules (Fig. 3, Table 1). This indicates a density-dependent retardation of nitrogen fixation in excess of a reduction of plant growth.

C. nutans had no detectable effect on nodulation or acetylene reduction. Although *C. nutans* seedlings may occur at very high densities in the field in the first few months after germination (Wardle et al., 1992, 1993a,b), they appear to exert very little interference against legumes (or, presumably, other pasture components), and *T. repens* seedlings only appear sensitive to other plants of its own species.

Additive experiment

The small rosette *C. nutans* plants sampled on 2 September 1991 did not exert statistically significant effects on any of the *T. repens* variables measured (data not presented), reinforcing the conclusion of the mixture experiment that seedling thistles do not directly interfere with *T. repens* growth and nitrogen fixation.

Table 3. Ethylene produced in acetylene-reduction bioassay (μmol/h/dm^2) in soil at varying distances from *Carduus nutans* plants, in response to above-ground and below-ground interference (additive experiment)

Developmental stage of *C. nutans*	Nature of interference from *C. nutans*	Distance from *C. nutans*			
		6 cm	12 cm	20 cm	28 cm
Rosette (vegetative) (11 December 1991)	Root + shoot	21.5b	25.2a	25.4a	23.9a
	Shoot	26.1a	25.6a	24.4a	23.9a
	Root	21.7b	24.6a	25.6a	23.3a
	None	28.2a	26.2a	26.0a	24.2a
Flowering (annual plants) (5 February 1992)	Root + shoot	9.7b	11.6a	13.8a	14.0a
	Shoot	10.4b	12.5a	14.2a	14.0a
	Root	12.4ab	13.6a	15.0a	15.0a
	None	14.1a	14.7a	14.0a	15.0a
Flowering (biennial plants) (3 December 1992)	Root + shoot	4.9c	4.7b	7.2ab	8.6a
	Shoot	5.2c	5.0b	5.2b	8.3a
	Root	7.9b	8.6a	8.3a	8.8a
	None	9.1a	9.0a	8.4a	8.7a

Within each developmental stage × distance combination, numbers followed by different letters are significantly different at $p = 0.05$ (least significant difference test).

The root weight of *T. repens* (data not presented), nodule density (Table 2) and acetylene reduction on an area basis (Table 3) were adversely affected by interference effects from large rosette and flowering *C. nutans* plants. The large rosette plants only suppressed these variables by root interference, and these were only detectable in the innermost arc. This is the stage when the *C. nutans* plants are most rapidly acquiring resources for flowering, and the stage when root competition is likely to be most intense. The flowering plants, however, only suppressed these variables when above-ground interference was permitted, and this extended either to the innermost arc (annual *C. nutans* plants) or three arcs (biennial *C. nutans* plants). This interference occurred only after the rosette leaves had died, and it is unlikely that light interference from *C. nutans* was suppressing the *T. repens* plants at this stage. Shading by *C. nutans* is characteristically greatest at the large rosette stage (Popay and Medd, 1990; Wardle *et al.*, 1993a), i.e. when shoot interference effects against *T. repens* was not detected. Therefore allelopathy was probably operating and this conclusion is supported by our previous results which showed that suppression of legumes by the allelopathic effects of decomposing rosette leaves is an important interference mechanism exerted by *C. nutans* (Wardle *et al.*, 1993a,b).

Reduction of nodule density and acetylene reduction on an area basis appears to be largely related to the reduction of root biomass. However, *T. repens* nodule density per g root weight was also suppressed in the innermost arc by shoot interference of biennial flowering *C. nutans* (Table 4). This suggests that nodulation ability of *T. repens* roots is sensitive to allelopathic influences of *C. nutans*. The sensitivity of nodulation to allelochemicals has been previously demonstrated by Rice (1964, 1965) and Blum and Rice (1969) but this aspect has received relatively little attention since then, especially since the criticism levelled against allelopathy by Harper's (1977) book (see Williamson, 1990).

Field experiment

The field study revealed that *C. nutans* has the potential to vastly modify the pasture species composition (Fig. 4). Productivity of the weedy monocotyledonous plants and *L. perenne* was initially inhibited in the thistle rosette (W) zone relative to the control pasture (P) zone. However, significant stimulation of these species was observed in the W zone on 17 February 1993, pos-

Table 4. *Trifolium repens* nodule density (numbers per g dry weight roots) at different distances from flowering *Carduus nutans* plants, in response to above-ground and below-ground interference (additive experiment)

Developmental stage of *C. nutans*	Nature of interference from *C. nutans*	Distance from *C. nutans*			
		6 cm	12 cm	20 cm	28 cm
Flowering (*annual plants*)	Root + shoot	477a	470a	419a	434a
(5 February 1992)	Shoot	457a	452a	400a	464a
	Root	415b	410a	419a	443a
	None	464a	388a	414a	455a
Flowering (*biennial plants*)	Root + shoot	187a	252a	210b	226a
(3 December 1992)	Shoot	148b	197b	270a	228a
	Root	201a	219a	214b	222a
	None	203a	210a	219b	237a

Within each developmental stage × distance combination, numbers followed by different letters are significantly different at $p = 0.05$ (least significant difference test).

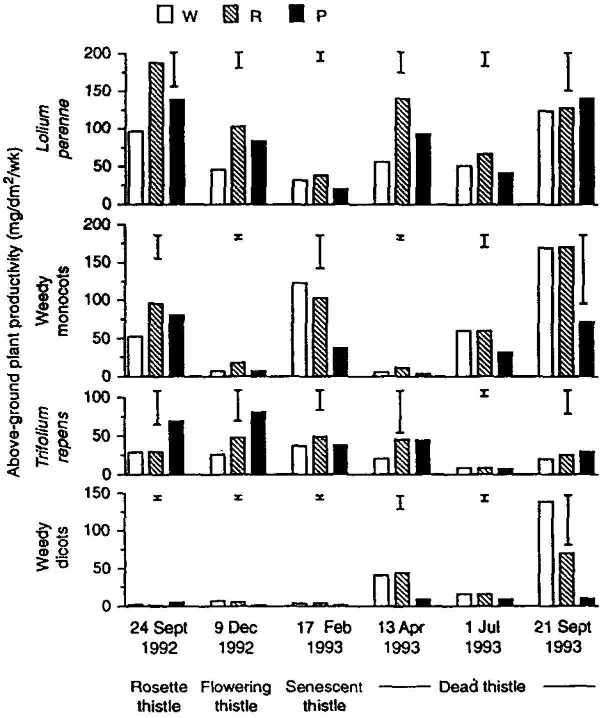

Fig. 4. Above-ground primary productivity in areas occupied (or previously occupied) by thistle rosette (W), in 10 cm wide rings surrounding the rosette (R), and in 0.5 m × 0.5 m areas of control pasture (P). Vertical bars represent the least significant difference ($p = 0.05$) for each sampling time.

sibly because the shading provided by the flowering *C. nutans* plants provided a superior microclimate for plant growth. Growth of monocotyledonous plants was also stimulated in the 10 cm ring (R) zone throughout much of the experiment. Meanwhile *T. repens* growth was significantly inhibited by *C. nutans* for the first two sampling dates. This supports the observations of Wardle et al. (1993a,b) that legumes are more susceptible than grasses to interference effects from *C. nutans*, and that *C. nutans* may shift the competitive balance between clovers and grasses, in favour of the latter. From 13 April 1993 onwards, the ingress of monocotyledonous weeds was highest in the W and R zones, probably because gaps created by suppression of other pasture components (especially *T. repens*) were conducive to invasion by these plants (Panetta and Wardle, 1992). Similar patterns were found for the dicotyledonous weeds on 13 April and 21 September 1993.

Acetylene reduction was severely reduced in the W and R zones, from 9 December 1992 until 1 July 1993 (Fig. 5). This inhibition is greatly in excess of reduction of *T. repens* productivity; for example *T. repens* growth was unaffected by *C. nutans* on 17 February 1993 while acetylene reduction was reduced by 76%. It would have been desirable to assess acetylene reduction in relation to *T. repens* root biomass (rather than shoot growth) as was done in the additive experiment, but this is not feasible in a multi-species mixture. The nitrogen-fixing ability of *T. repens* appears to be retarded for at least four months after the *C. nutans* plant has died. *C. nutans* also severely suppressed nitrogen fix-

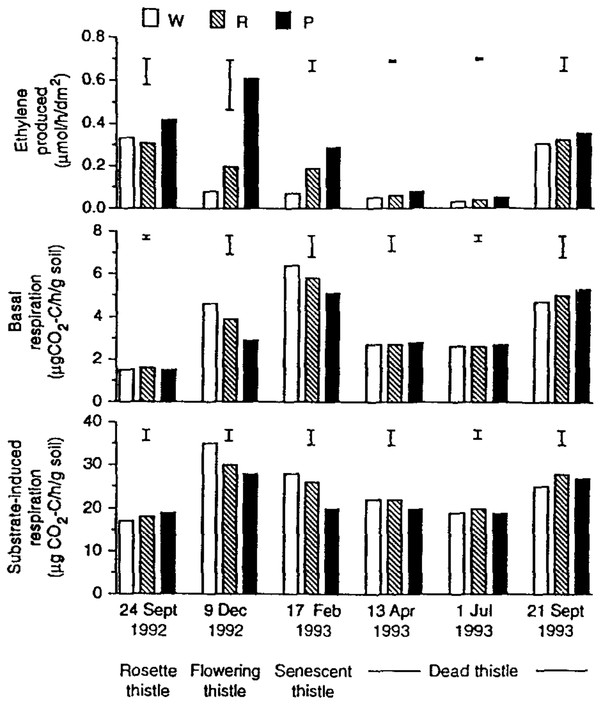

Fig. 5. Acetylene reduction, basal and substrate-induced respiration in areas occupied (or previously occupied) by thistle rosettes (W), in 10 cm wide rings surrounding the rosette (R), and in 0.5 × 0.5 m areas of control pasture (P). Vertical bars represent the least significant difference ($p = 0.05$) for each sampling time.

ation in the early summer when nitrogen fixation is otherwise maximal, suggesting that the patch effects created by thistles have the potential to greatly alter input of soil nitrogen. These patches are coincident with the dimensions of the large rosette plants, which had a mean diameter (±SD) of 64 ± 10 cm in the present study.

The glucose-responsive microbial biomass (estimated using S.I.R.) and soil basal respiration are both sensitive to the available nitrogen status in soil (Wardle, 1992). In the present study these variables were stimulated in the W zone on 9 December 1992 and 17 February 1993, but were otherwise unaffected by *C. nutans* (Fig. 5). This stimulation is likely to result from the substrate added in decomposing large rosette leaves, or reduced competitive effects of the other pasture components in the W zone (Okano et al., 1989, 1991).

Consequences for nutrient input

The three experiments in combination suggest that *C. nutans* does not exert strong effects against *T. repens* growth, nodulation or acetylene reduction, until the rosette plant begins to produce flower-heads and the rosette leaves decompose. At this stage, reduction of root growth (additive experiment) and shoot growth (field experiment) becomes detectable, and this is accompanied by a considerable reduction in nodule density and nitrogen fixing ability. These effects were most pronounced in the field, probably because the *C. nutans* plants grew faster and to a larger size, properties which are frequently associated with superior competitive ability (Grime, 1973; Gaudet and Keddy, 1988). In all experiments, the decline in nodulation or acetylene reduction induced by interference from other plants was frequently greater than the reduction observed in clover growth rate or biomass, and in some cases this discrepancy was substantial. Previous studies have shown that rhizobia population growth, legume nodulation density and nodule colouration may all be adversely affected by interference phenomena, and allelopathy has frequently been implicated (e.g. Murthy and Ravindra, 1974; Murthy and Nagodra, 1977; Sarma, 1983; Rice, 1992).

Other mechanisms proposed for explaining suspected instances of allelopathic interference (Harper, 1977) can be effectively excluded. It is possible that nitrogen fixation was partially reduced by enhanced microbial build-up and associated nutrient immobilisation, but since this build-up was not substantial in the W zone (especially when compared with changes in microbial biomass that can occur in some ecosystems: Wardle et al., 1992), it is unlikely that this mechanism would have been responsible for the extreme reductions observed in acetylene reduction in our study. Soil moisture levels did not differ markedly between the W, R and P zones in the field (data not presented). Shading by *C. nutans* is unlikely to explain much of the observed trends, because reduction of light intensity was greatest when the rosette leaves were present, and inhibition of nodulation and acetylene reduction was observed only after these leaves had died. Root interference effects from *C. nutans* were not significant when plants were in full flower (additive experiment) making root competition effects unlikely. No evidence was observed for greater herbivory in the W than P zones in the field, and herbivory was almost nonexistent in the additive experiment (cf. Bartholomew, 1970). Mineral nitrogen release from decomposing rosette leaves may have contributed slightly to reduced nitrogen fixation, but corresponding glasshouse experiments suggest that this effect is likely to be transient and probably insignificant. Furthermore, these severe interference effects against acetylene reduction were not observed in a separate experiment under plants of *Senecio jacobaea* L., an annual/biennial invasive species with a similar growth habit occupying a similar niche in the Waikato region (Wardle et al., unpublished data).

C. nutans is highly effective at promoting recruitment of its own species once it is established in pasture. As rosette tissues decompose, gaps are created, and *C. nutans* seedlings are favoured in the area that the parent plant previously occupied (Wardle et al., 1993b). In the field experiment, the number of thistle seedlings per m^2 (mean ±SD) at 21 September 1993 in the W, R and P zones were 91 ± 47, 52 ± 29 and 5 ± 6 respectively. This helps *C. nutans* to operate as a successful weed, since when established, it has the potential to rapidly become a dominant component of the pasture flora. The effects of *C. nutans* on forage legumes is particularly severe, and appears to involve allelopathy (Wardle et al., 1993a). *C. nutans* thus has the ability to create conditions which are unsuitable for *T. repens* establishment, persistence and ultimately nitrogen fixation, particularly when present in high numbers. Agronomic practices which encourage grasses may be effective at supressing *C. nutans*, which may in turn be beneficial in encouraging forage legumes (Wardle et al., 1992, 1994). Vitousek and Walker (1989) demonstrated that invasive plant species can create conditions conducive for soil nitrogen build-up in terrestrial ecosystems. We suggest that the reverse can also occur, and invasive species such as *C. nutans*, which create environments favouring regrowth of its own seedlings (thus creating a positive feedback situation) have the potential to induce long-term reductions of soil nitrogen input.

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