Responses of individual *Arabidopsis thaliana* plants to changes in the number and spatial distribution of conspecific neighbours.

CHRISTOPHER A. KIRKBY
Department of Biology, University of York, Heslington, York YO10 5DW, UK

Summary

1 The influence of two neighbourhood predictors of plant performance, number and spatial distribution of neighbours, on the dry mass of individual *Arabidopsis thaliana* plants was studied under controlled conditions.

2 Target plants were grown in association with one (control) or two (treatment) conspecific neighbours. The dispersal pattern of neighbours in treatments varied although the distance between target and neighbours was constant. Dry mass, box area and number of leaves of each individual were assessed after 32 days of growth.

3 Dry mass of target plants with two neighbours was found to be 42% lower than those with only one neighbour. The dispersal pattern of neighbours accounted for 14% of variation in the dry mass of target individuals. The relative position of plants after 32 days varied relative to their sown position. In treatments, the dry mass of the smallest neighbour could be described by a factor of the distance to its largest neighbour.

4 A parallel calibration experiment revealed that box area and number of leaves of an individual were good predictors of dry mass. Comparisons of relative growth rate of targets between treatments revealed that competition affects growth only during the early stages of plant development.

5 Results emphasize the usefulness of experimental manipulation to untangle the relative importance of neighbourhood parameters which mediate the strength of competition felt by individual plants.

Key-words: *neighbourhood, intra-specific competition, annual plants, relative growth rate.*

Correspondence: chris_kirkby@yahoo.com

Introduction

The structure and dynamics of plant communities is governed by competition between individuals. Competition is defined as the interaction between neighbouring plants induced by the necessity to share limited resources, leading to a reduction in survivorship and/or growth. Systematic studies to isolate and understand the principles underlying competition at the individual level began in the 1950s (Hozumi, Koyama & Kira 1955, Yoda, Kira & Hozumi 1957). Recent research of natural and experimental communities has shown that the most effective way of understanding competition is to study it from the point of view of a focal or target plant and the characteristics of its perceived neighbourhood (Mahdi & Law 1987, Law *et al*. 2000). A neighbourhood can be described as the area about a plant circumscribing all other individuals which interact with the plant (Silander & Pacala 1985b). Studies of plant neighbourhoods have identified a set of competitively active components including the number, age, size, distance to, genotype, species, emergence time, movement, and spatial distribution of neighbours (Mack & Harper 1977, Weiner 1982, Silander & Pacala 1985a, Firbank & Watkinson 1987, Bergelson 1993, Molofsky 1999). The effects of many of these can be described in algorithmic form, and in turn used to build individual or neighbourhood-based computer models that mimic the dynamics of plant communities (Firbank & Watkinson 1985a, Silander & Pacala 1985b, Czárán & Bartha 1992, DeAngelis & Gross 1992, Ellison, Dixon & Ngai 1994, Miller & Weiner 1989, Law *et al*. 2000).

This paper is concerned with identifying from first principles the relative importance of two neighbourhood predictors of plant performance including, number and spatial distribution of neighbours from the point of view of individual plants using a simple experimental approach. Small-scale movement of individuals experiencing competition is also quantified. I also illustrate the usefulness of plant size characteristics such as box area and number of leaves as predictors of dry mass in rosette-forming plants. The aims were to test the following specific null hypotheses: **1** In a community of two plants there is no effect on relative growth rate or dry mass after a set period if a third plant is added to the community; **2** Given that a target plant has two neighbours, which are equidistant from the target, the inter-neighbour distance or angular dispersion has no effect on the relative growth rate or dry mass of the target after a set period; **3** The relative position of plants does not change over a set period; **4** There is no relationship between dry mass of a plant and variables such as box area and number of leaves.

The plant species chosen for this controlled experimental study of competition was *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) or thale cress, a weedy annual (genotype "Colombia

wildtype"). This plant was considered ideal for investigating intra-specific competition in lieu of characteristics, such as: fast growth rate, generation times as short as four to six weeks (Silander & Pacala 1985a); more or less symmetrical circular rosette morphology (Silander & Pacala 1985a, Ellison et al. 1994, Bowman 1994); little or no genetic variation within lines; rapid and near synchronous germination; as well as the plethora of background information available describing its morphology, physiology and ecology (Bowman 1994, Myerscough & Marshall 1973).

Methods

The study was conducted between November and December 2000. A total of 180 square pots (130 x 130 x 130 mm) were filled with Levington F2S standard potting compost, watered, doused with a soil based residual insecticide (Intercept®) and placed under artificial light (16 hour-day) in a temperature-controlled greenhouse at the University of York, North Yorkshire, UK. The experimental design included a control, representing a community of two plants (one target and one neighbour); and five treatments (A - E), each representing a community of three plants (one target and two neighbours, Fig. 1). The only factor that varied between treatments was the distance between neighbours at time of sowing (A = 2 mm, B = 4 mm, C = 7 mm, D = 11 mm, E = 16 mm), which in turn was proportional to the angular dispersion of neighbours (A = 14^{o}, B = 29^{o}, C = 52^{o}, D = 87^{o}, E = 180^{o}). Thirty pots were randomly assigned to the control and to each of the five treatments.

On the 9th of November 2000, five seeds were sown at each predetermined neighbour location in all 180 pots (Fig. 1). Six days later, when neighbours were at the two-cotyledon stage, the definitive individuals were identified. In the case of treatments this was based on distance between individuals and equality of size. All other seedlings were eliminated. Immediately following this, five target seeds were sown at a point 8 mm away from each neighbour (Fig. 1). From this point on time was measured relative to this day. On day 6, the definitive target seedling was identified and others eliminated. This lag period between sowing the neighbours and target plants eliminated the random chance of targets attaining a rank size greater than their neighbours, and thus reduced an element of variation in final target dry mass. From the moment the neighbours were seeded pots were watered daily. On a weekly basis the positions of pots relative to the light source was altered in a random fashion to ensure even exposure of plants to available light intensity. It was assumed that edge effects were negligible.

On day 8, three variables were measured: distance between plants to the nearest 0.01 mm, measured from the centre of rosettes; box area of target, measured to the nearest 0.01 mm^2 (Fig. 2); and number of leaves (>1 mm in length) per target. The latter two variables were subsequently reassessed every four to eight days. On day 32, all three variables were assessed for a final time. Following this, all plants were harvested at ground level, placed in wax-proof envelopes and dried in an oven at 70^{o}C for at least 48 hrs. They were subsequently weighed to the nearest 0.001 g.

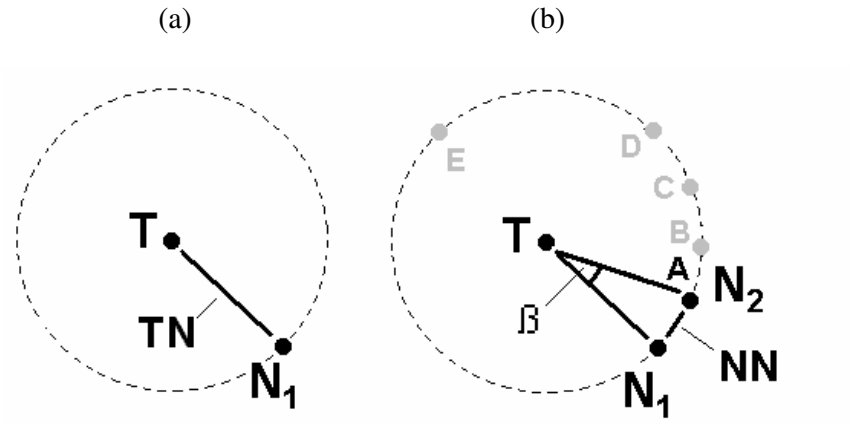

Fig. 1 Schematic diagram showing planting arrangement in (a) control and (b) treatment pots. T = target plant, N_1 = neighbour, NN = neighbour separation distance (A = 2 mm, B = 4 mm, C = 7 mm, D = 11 mm, E = 16 mm), TN = target-neighbour distance (8 mm), ß = angular dispersal of neighbours (A = 14^{o}, B = 29^{o}, C = 52^{o}, D = 87^{o}, E = 180 ^{o}).

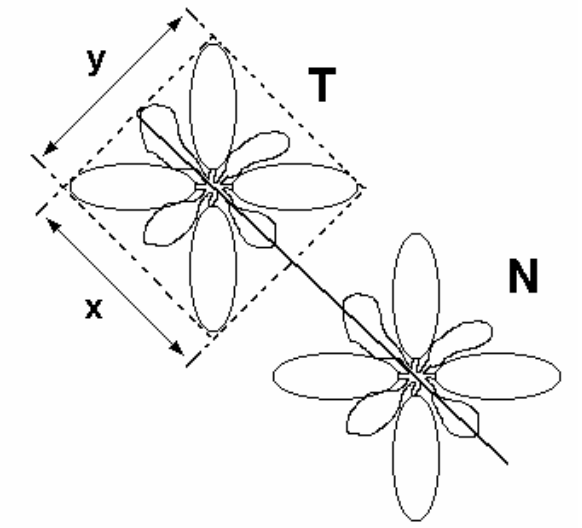

Fig. 2 Schematic representation of the box area of a target plant (T), depicting the two variables (x, y) measured. N = neighbour.

CALIBRATION

A parallel calibration experiment was undertaken to parameterise the relationship between alternative target size characteristics (box area and number of leaves) and dry mass. Eighty one pots were prepared and sown in a manner similar to the control, such as to leave two individuals per pot, one target and one neighbour. After the target was 8 days old, and for every two to four days after that until targets were 29 days old, 7 pots were chosen at random and each plant was measured for: box area; number of leaves; and dry mass as before. In this way a gradual series of plant size and dry mass measurements were generated spanning a growth period of 5 weeks. The developmental stages sampled in this way extended from seedling to early inflorescence. Data on dry mass, box area and number of leaves was collected for 128 calibration plants.

STATISTICAL ANALYSIS

To test for significant differences in target dry mass, one-way ANOVA and post-hoc tests or equivalent non-parametric tests were utilised. Changes in the positions of plants during the course of the experiment, relative to their starting point, was investigated using t-tests and one-way ANOVA. To calibrate the relationship between plant size characteristics (box area and number of leaves) and dry mass, it was necessary first to Log transform the dry mass and box area data in order to normalise their distributions. Linear regressions were then used to determine which of the two characters was the best predictor of dry mass. Further ANOVA techniques were subsequently used to investigate changes in relative growth rate of target plants at different stages of development based on the best predictor.

Results

LATERAL DISPLACEMENT OF SHOOT

Mean neighbour separation distance increased significantly over the course of the experiment (Fig. 3a, ANOVA, $F_{4,144} = 548.65$, $P < 0.001$). The magnitude of this lateral displacement effect was strongest where neighbour separation distance was shortest. With respect to target-neighbour distance, a similar significant trend during the course of the experiment was also observed (Fig. 3b, Kruskal-Wallis, $X^2 = 31.85$, $P < 0.001$). As a consequence, the final angular dispersion values for three of the treatments (A, B and E) varied significantly from those anticipated (Fig. 3c, Kruskal-Wallis, $X^2 = 128.65$, $P < 0.001$).

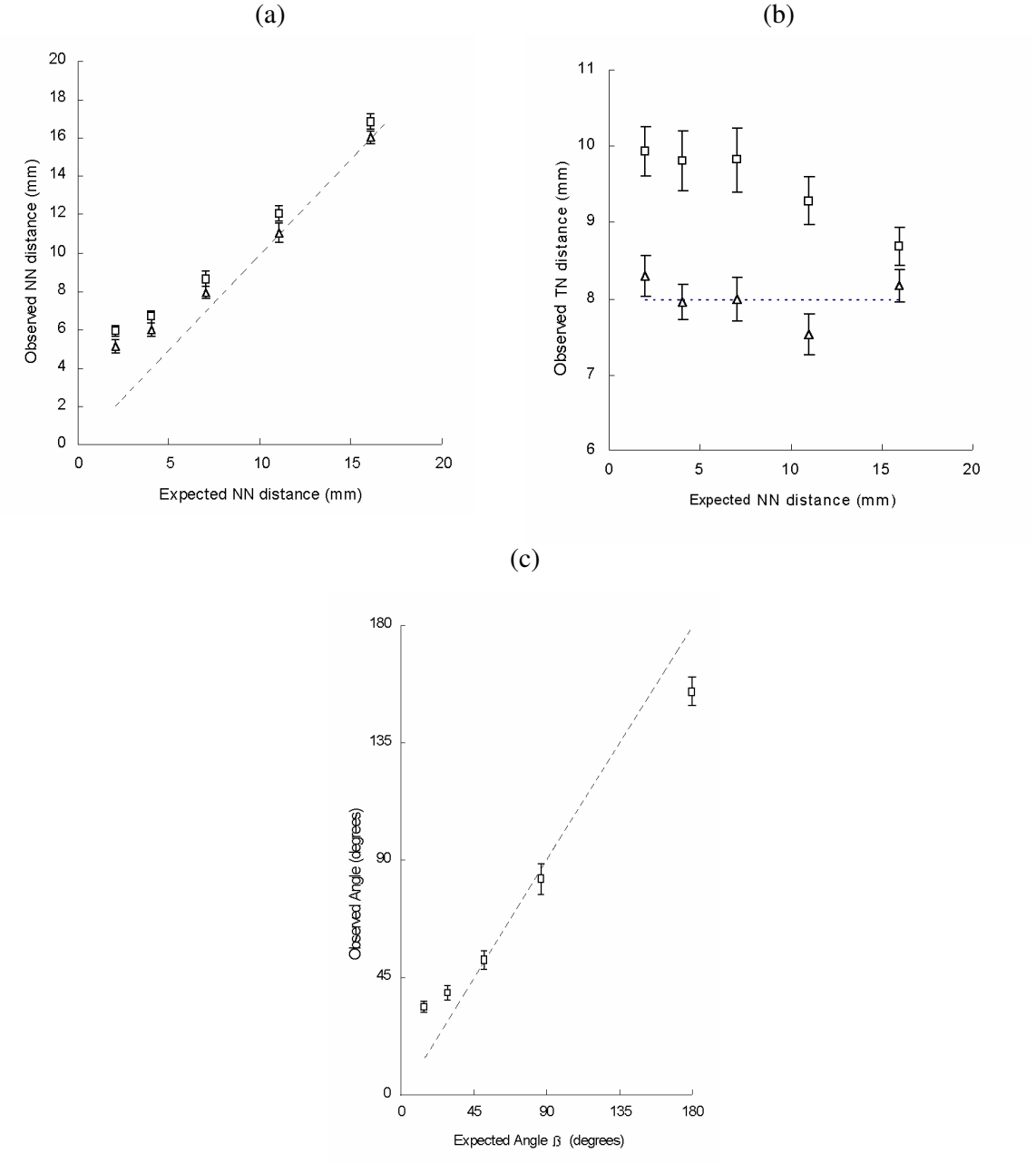

Fig. 3 Lateral displacement of plants from expected positions. (a) Neighbours (NN distance), (b) Targets (TN distance), (c) Angular dispersion (ß). Dotted lines = expected, triangles = observed day 8, squares = observed day 32. Bars denote +/- 95% CI.

CALIBRATION

There was a strong positive linear relationship between box area and dry mass (Regression, F = 1,262.6, $P < 0.001$, $r^2 = 0.91$)(Fig. 4). The equation for the relationship was y = 0.979x – 5.12,

where y = dry mass and x = box Area. This relationship was virtually identical to that calculated for the 178 target plants in the competition experiment (Fig. 4): y = 0.979x – 5.21. The trend between number of leaves and Log_{10} dry mass was also positive and linear (Regression, F = 1,184.7, $P < 0.001$, $r^2 = 0.90$). The r^2 value, however, was marginally lower than for box area. Box area accounts for a greater amount of variation in dry mass and is therefore a better predictor of dry mass.

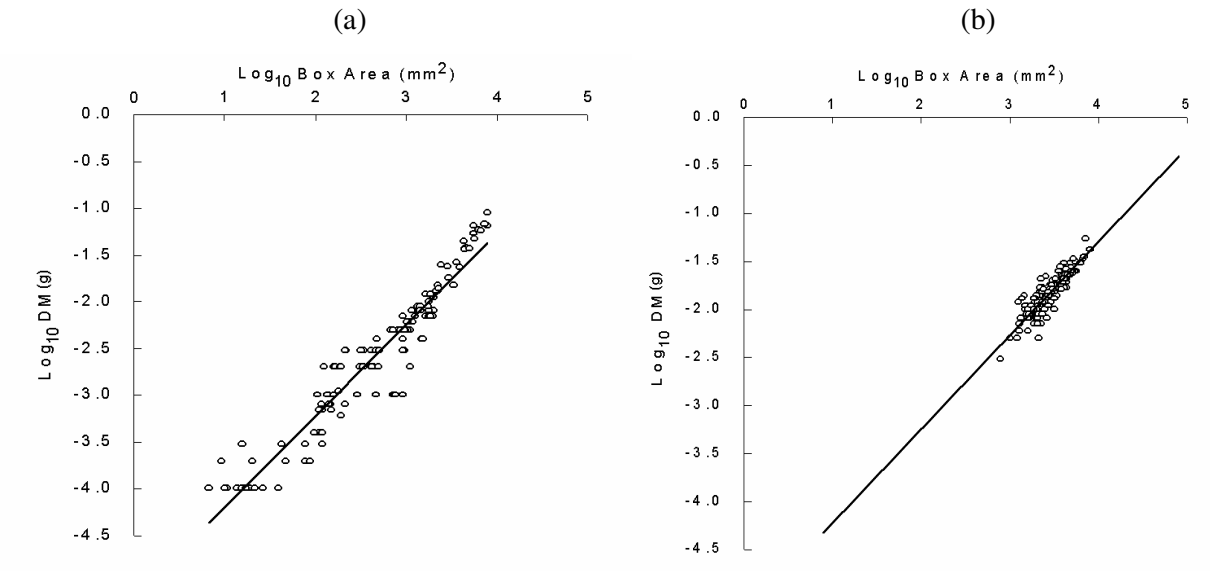

Fig. 4 Relationship between box area and dry mass. (a) All calibration plants (n = 128), (b) Competition target plants (n = 178).

COMPETITION

Number of neighbours

Target plants with two neighbours had a dry mass 42% lower than those with one neighbour (Fig. 5, Mann-Whitney, Z = -6.06, $P < 0.001$). On day 8, relative growth rate of targets with two neighbours was significantly lower than those with one neighbour (Fig. 8, ANOVA, $F_{1,176} = 11.23$, $P < 0.001$), although this trend broke down after day 16 and was not re-established. Mean dry mass of the smallest neighbour exhibited the same pattern as target plants (Mann-Whitney, Z = -6.99, $P < 0.001$). The reduction in dry mass in this case was 41%.

Spatial distribution of neighbours

The dry mass of target plants differed significant between treatments (Fig. 6, ANOVA, $F_{4,144} = 3.24$, $P < 0.05$). The relationship was parabolic such that, as angular dispersion of neighbours increased from $0 – 180^0$ so target dry mass decreased. On day 8, relative growth rate of targets varied significantly between treatments (Fig. 8, ANOVA, $F_{4,144} = 9.55$, $P < 0.001$), although

as before this trend broke down after day 16 and was not re-established. Relative growth rate of targets fell by approximately 45% between days 16 and 32. Dry mass of the smallest neighbour did not vary between treatments (Fig. 7, ANOVA, $F_{4,144} = 1.49$, $P = 0.20$), nor did the combined dry mass of the two neighbours.

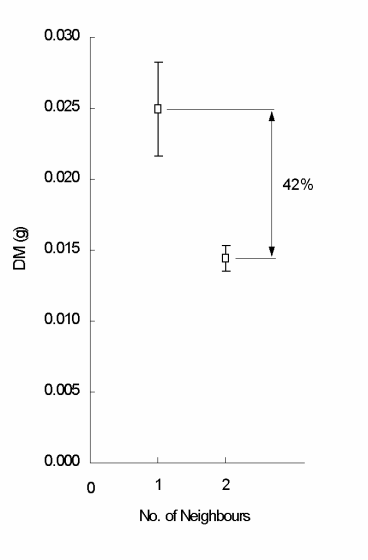

Fig. 5 Dry mass of target plants grown in association with one or two neighbours. Bars denote +/- 95% CI.

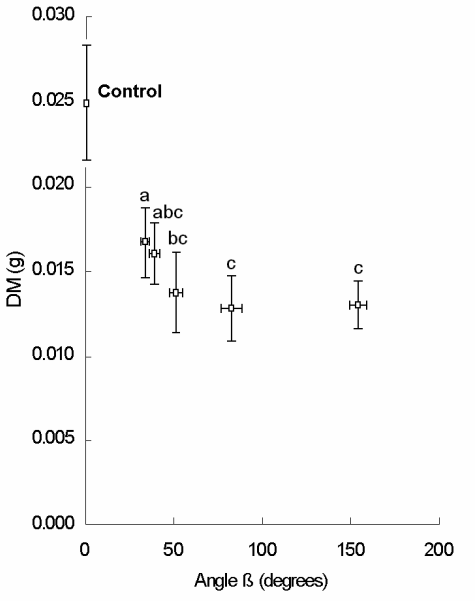

Fig. 6 Relationship between dry mass of targets and angular dispersal of neighbours. Treatments sharing a letter did not differ significantly from each other at the 0.05 significance level. Bars denote +/- 95% CI.

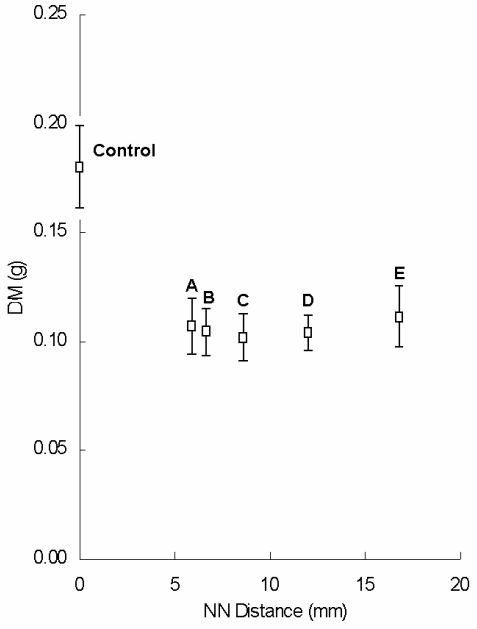

Fig. 7 Relationship between dry mass of smallest neighbour and neighbour separation distance (NN). Letters denote treatments. Bars denote +/- 95% CI.

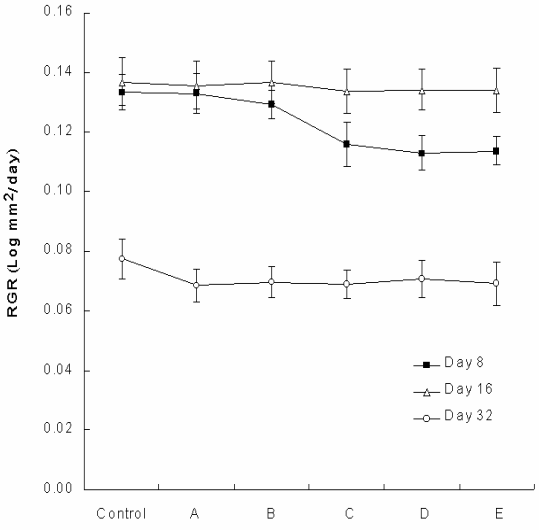

Fig. 8 Relative growth rate (RGR) of targets, based on box area, at discrete times during the experiment. Black squares = day 8, triangles = day 16, circles = day 32. Bars denote +/- 95% CI.

Discussion

Increasing the number of competitors around a plant clearly has a marked negative impact on dry mass, thus the first null-hypothesis can safely be rejected. The magnitude of the effect felt by both the target and smallest neighbour was very similar (~40%) underlining the significance of this factor. This type of first-order effect is in accordance with findings from other studies of intra- and inter-specific competition (Mack & Harper 1977, Firbank & Watkinson 1985b, 1987, Pacala & Silander 1990, Bergelson 1993, Weiner, Kinsman & Williams 1998), and the fact that the smallest neighbour was also affected to a similar extent as the target, in relative terms, supports the widely-held idea that a neighbour plant of a given size causes a given percentage reduction in the size of a focal plant, regardless of the size of this plant (Silander & Pacala 1985a, 1985b, Firbank & Watkinson 1987, Pacala & Silander 1990, Ellison, Dixon & Ngai 1994).

Results indicate that spatial distribution of neighbours about a target accounted for a significant variation in target dry mass and is thus an important factor mediating the strength of competition felt by an individual, a finding also reported by (Silander & Pacala 1985a, Weiner, Kinsman & Williams 1998), but contrary to that expressed by Firbank & Watkinson (1987). Spatial distribution should therefore be considered when constructing and testing models of plant competition. Indeed, other authors (Mack & Harper 1977, Waller 1981, Bergelson 1993) also state that terms that describe the dispersion of plants improve model fit. The possible mechanisms underlying the observed effect of spatial distribution of neighbours on a target in this particular experiment are two-fold. Firstly, when the angle to neighbours is small the competitive forces neighbours exert on the target are relatively unidirectional. In terms of occupying space, the target could extend its roots, and have its shoot toppled, in the direction of least interference. This habit however could be compromised when forces of interference come from opposite directions. This type of interaction is in line with zone of influence models such as those described by DeAngelis & Gross (1992) and Czárán & Bartha (1992). Secondly, when neighbours are closer to each other than to the target the degree of mutual interference they exert on each other may be enough to release the target plant from some of the constraints of competition. This phenomenon of competitive release is described in other studies (Hozumi, Koyama & Kira 1955, Yoda, Kira & Hozumi 1957, Franco & Harper 1988) and can be described by distance-based models (DeAngelis & Gross 1992, Czárán & Bartha 1992, Law *et al.* (2000). However, results indicate that the dry mass of the smallest neighbour was not strongly related to the distance away from its companion neighbour, as might have been expected, although the sign of the relationship was correct (+). There are three explanations for the absence of a distance decay relationship to account for the pattern of neighbour dry mass observed:

1 When neighbour separation distance is greater than target-neighbour distance (as is the case in treatments D & E), the target plant may exert enough competitive pressure on the smallest neighbour to reduce its dry mass; **2** The developmental stage of neighbours may have been different to that observed in other studies involving *A. thaliana* (Law *et al.* 2000); **3** Insufficient replication.

It is clear from the results of this experiment that *A. thaliana* stems are prone to lateral displacement or toppling even at plant separation distances in excess of 16 mm. This trend is particularly noticeable where plant separation distances are short, as found by Silander & Pacala (1985a). The pattern of lateral movement shown by both targets and neighbours is consistent with displacement in the direction of least resistance. To what degree this phenomenon may involve active movement is difficult to say, however it is most likely that the causal mechanism is passive and mediated by physical forces exerted by lateral growth of the plants. The presence of trichomes on the upper and lower surface of leaves (Bowman 1994) increases friction and so may help to mediate this effect. Silander & Pacala (1985a) have already shown that accounting for such movement can improve the fit of neighbourhood models. This variable should be more widely investigated and applied in future studies of plant competition, for it is commonly assumed in many models that a plant's position in a community is constant.

This study shows that, given an adequate calibration study can be undertaken, the dry mass of competing plants at the seedling to flowering stage can be accurately predicted from box area measurements. Box area, therefore, can effectively be used to estimate relative growth rate for individual plants without requiring destructive sampling. The relative growth rate results in this experiment clearly indicate that the competitive interference observed between plants is felt most strongly at the seedling stage, implying that final dry mass is set early-on in the life of a plant, a conclusion also described by Harper (1977) and Firbank & Watkinson (1987). This would suggest that future competition studies of this type could be undertaken over shorter time periods, as dry mass or box area after 16 days (~2 weeks) is a good indicator of final dry mass at inflorescence.

In order to identify an algorithm to fully describe the relationship of the effect of number of neighbours in the neighbourhood competition paradigm, and thus use this in the generation or improvement of computer models, it will be necessary to repeat this experiment using increasing numbers of neighbours. Also, by varying the lag period between the dates the neighbours and target plants are sown the effect of emergence time and size of neighbours can be determined.

Acknowledgements

I would like to thank Richard Law and Drew Purves who provided useful comments to the issues discussed here, and Colin Abbot and Christopher Lancaster for their help in the greenhouse. The Natural Environment Research Council (NERC) provided the author with a studentship to attend the MRes course in Ecology and Environmental Management at the University of York, of which this investigation is part.

References

Bergelson, J. (1993) Details of local dispersion improve the fit of neighbourhood competition models. *Oecologia*, **95**, 299-302.

Bowman, J. (1994) *Arabidopsis: an atlas of morphology and development*. Springer-Verlag, New York.

Czárán, T. & Bartha, S. (1992) Spatiotemporal dynamic models of plant populations and communities *Trends in Ecology and Evolution*, **7**, 38-42.

DeAngelis, D.L. & Gross, L.J. (1992) *Individual-based models and approaches in Ecology*. Chapman & Hall, New York.

Ellison, M.A., Dixon, P.M. & Ngai, J. (1994) A null model for neighbourhood models of plant competitive interactions. *OIKOS*, **71**, 225-238.

Firbank, L.G. & Watkinson, A.R. (1985a) A model of interference within plant monocultures. *Journal of Theoretical Biology*, **166**, 291-311.

Firbank, L.G. & Watkinson, A.R. (1985b) On the analysis of competition within two-species mixtures of plants. *Journal of Applied Ecology*, **22**, 503-517.

Firbank, L.G. & Watkinson, A.R. (1987) On the analysis of competition at the level of the individual plant. *Oecologia*, **71**, 308-317.

Franco, M. & Harper, J.L. (1988) Competition and the formation of spatial pattern in spacing gradients: an example using *Kochia scoparia*. *Journal of Ecology*, **76**, 959-974.

Hozumi, K., Koyama, H. & Kira, T. (1955) Intraspecific competition among higher plants. IV. A preliminary account on the interaction between adjacent individuals. *Journal of the Institute Polytechnic Osaka City University*, **6**, 121-130.

Law, R., Purves, D.W., Murrell, D.J. & Dieckman, U. (2000). Causes and effects of small-scale spatial structure in plant populations. In the proceedings from the B.E.S. Symposium entitled “Plants stand still but their genes don’t”, in press.

Mack, R.N. & Harper, L. (1977) Interference in dune annuals: spatial pattern and neighbourhood

effects. *Journal of Ecology*, **65**, 345-363.

Mahdi, A. & Law, R. (1987) On the spatial organisation of plant species in a limestone grassland community. *Journal of Ecology*, **75**, 259-476.

Molofsky, J. (1999) The effect of nutrients and spacing on neighbour relations in *Cardamine pensylvanica*. *OIKOS*, **84**, 506-514.

Myerscough, P. J. & Marshall, J.K. (1973) Population dynamics of *Arabidopsis thaliana* (L.) Heynh. Strain "estland" at different densities and nutrient levels. *New Phytologist*, **72**, 595-617.

Pacala, S.W. & Silander, J. A. (1990) Tests of neighbourhood population dynamic models in field communities of two annual weed species. *Ecological Monographs*, **60**, 113-134.

Silander, J. A. & Pacala, S.W. (1985a) Neighbourhood predictors of plant performance. *Oecologia*, **66**, 256-263.

Silander, J. A. & Pacala, S.W. (1985b) Neighbourhood models of plant population dynamics: single-species models of annuals. *The American Naturalist*, **125**, 385-412.

Waller, D.M. (1981) Neighbourhood competition in several violet populations. *Oecologia*, **51**, 116-122.

Weiner, J. (1982) A neighbourhood model of annual-plant interference. *Ecology*, **65**, 1237-1241.

Weiner, J., Kinsman, S. & Williams, S. (1998) Modelling the growth of individuals in plant populations: Local density variation in a strand population of Xanthium strumerium (Asteraceae). *American Journal of Botany*, **85**, 1638-1645.

Yoda, K., Kira, T., & Hozumi, K. (1957) Intraspecific competition among higher plants. IX. Further analysis of the competitive interaction between adjacent individuals. *Journal of the Institute Polytechnic Osaka City University*, **8**, 161-178.