ELSEVIER

Scientia Horticulturae 88 (2001) 97–106

SCIENTIA HORTICULTURÆ

www.elsevier.com/locate/scihorti

The effect of nitrogen on stolon and ramet growth in four genotypes of *Fragaria chiloensis* L.

Thomas J. Tworkoski[a,b,*], Thomas E. Benassi[b], Fumiomi Takeda[a]

[a]*United States Department of Agriculture, Agricultural Research Service (ARS), Appalachian Fruit Research Station, 45 Wiltshire Road, Kearneysville, WV 25430 9425, USA*
[b]*Department of Biology, Hood College, Frederick, MD 21701, USA*

Accepted 26 June 2000

Abstract

Plant foraging response is a process in which clonal plants proliferate in nutrient-rich sites by shortening stolon length and increasing ramet density. Conversely, stolon length increases and ramet density decreases in nutrient-poor sites. Four genotypes of strawberry (*Fragaria chiloensis* (L.) Duch.) were grown in a greenhouse for 10 weeks and treated with different concentrations of nitrogen. Genotypes differed in plant size, stolon and ramet production, and nitrogen distribution between parent and ramets. Genotype Q18 were the smallest plants with the greatest number of stolons and ramets, typical of the phalanx morphology. The other genotypes had fewer but longer stolons, typical of the guerrilla morphology. Number of stolons and ramet density increased with increased N more in Genotype Q18 than the other genotypes. Results indicate that vegetative growth changed in response to increasing N treatment of the parent plant by shortening the average stolon length, increasing the number of stolons, and increasing the number of ramets while maintaining total stolon length. Foraging response characteristics were observed in strawberry but varied among genotypes. Published by Elsevier Science B.V.

Keywords: *Fragaria*; Foraging response

* Corresponding author. Tel.: +1-304-728-2340; fax: +1-304-725-3451.
E-mail address: ttworkos@afrs.ars.usda.gov (T.J. Tworkoski).

0304-4238/01/$ – see front matter Published by Elsevier Science B.V.
PII: S0304-4238(00)00198-9

1. Introduction

Fragaria chiloensis L. is indigenous to the New World and ranges from Santa Barbara, CA, northward through western Alaska, and inland in coastal Chile and in the Andes Mountains (Hancock and Bringhurst, 1979). *Fragaria×ananassa* Duch., the cultivated strawberry, is thought to be derived from the hybridization of *F. virginiana* (L.) Duch. and *F. chiloensis* (L.) Duch. (Gleason and Cronquist, 1991). Populations of *F. chiloensis* L. in North America are found growing on coastal dunes containing 98% sand and low in moisture and nutrients, as well as woodland meadows with more fertile soils and higher moisture contents (Hancock and Bringhurst, 1979).

Reproduction in *Fragaria* is generally accomplished asexually by the production of stolons from the parent plant that can support several new plants (ramets). Sexual reproduction also occurs from seed, which is far less common. Plants that produce stolons have improved chances for progeny survival in harsh habitats (Silander, 1985). Stolons can develop clones that selectively exploit the environment. Clonal morphology has been categorized as ''guerrilla'' and ''phalanx'' (Lovett Doust, 1981). Guerrilla morphology is characterized by clonal plants that are widely dispersed on the stolon with extensive spreading. Phalanx morphology is characterized by clonal plants that are bunched closely together. *Fragaria* generally follow the guerrilla morphology.

Plants may produce stolons in a foraging response in suitable sites to ensure adequate nutrition (Birch and Hutchings, 1994; Cain, 1994; Kelly, 1994; Wijesinghe and Handel, 1994). Plant foraging response has been demonstrated using plants with stoloniferous growth habits such as *Glechoma hederacea* L. (Birch and Hutchings, 1994). By creating habitats which vary in the amounts of soil nutrients, Birch and Hutchings (1994) found that *G. hederacea* shorten stolon lengths and produce greater number of ramets on nutrient-rich sites. On nutrient-poor sites, some plants increased stolon length and decreased the number of ramets. However, other research found no change in clonal growth related to soil fertility (Gray and Call, 1993; Harnett, 1993). There is evidence that morphological plasticity of a species defines the foraging response to different soil fertilities (Alpert, 1991; Turkington and Klein, 1993; Cain, 1994; DeKroon and Hutchings, 1995).

Stolon length and number of ramets produced by strawberry plants are important to the strawberry industry, particularly the number of ramets a given cultivar can develop for the production of nursery and container plants (Bish et al., 1997; Hamann and Poling, 1997; Takeda, 1999). Nitrogen, in particular, can affect stolon length and can limit plant growth due to its solubility in water and high diffusion coefficient. The experiment was designed to determine strawberry plant (*Fragaria chiloensis* L.) stolon and growth response to different nitrogen concentrations and to evaluate intragenotypic variation of four strawberry genotypes.

2. Materials and methods

2.1. Plants

Seeds of four genotypes of *F. chiloensis* L. (labeled DNT, R07, Q18, and I19) were collected during a strawberry germplasm exploration (J.S. Cameron, pers. comm.). Stolon tips of these four genotypes were obtained from Washington State University, Vancouver, WA and were rooted in Pro-Mix BX (Premier Horticulture, Red Hill, PA) under intermittent mist in a greenhouse to generate plants of each clone. Plants were grown with natural sunlight with a 14 h photoperiod that was supplemented with high pressure sodium light (average mid-day intensity of 570 μmol s^{-1} m^{-2} PAR; 21 ± 3°C). Plants were placed in a cold chamber on December 18, 1995 to satisfy the chilling requirement. The chamber was maintained at 5°C with an 8 h photoperiod provided by fluorescent grow lights. The plants were removed from the cold chamber on January 25, 1996 and returned to the greenhouse. The next day the Pro-Mix was washed from the roots and the rooted stolon tips were transplanted into 13-cm plastic pots containing washed sand. Plants were watered as needed and one nitrogen treatment was applied to each plant.

2.2. Nitrogen treatments

Hoagland's solution with different nitrogen concentration was applied to each pot (Hoagland and Arnon, 1950). Nutrient concentrations were: 1 mM KH_2PO_4, 1 mM $MgSO_4{\cdot}7H_2O$, 1 mM $CaCl_2$, 0.008 mM H_3BO_3, 0.006 mM $MnSO_4{\cdot}H_2O$, 0.002 mM $ZnSO_4{\cdot}7H_2O$, 0.00008 mM $CuSO_4{\cdot}5H_2O$, 0.00006 mM $H_2MoO_4{\cdot}H_2O$, 0.009 mM $FeSO_4{\cdot}7H_2O$, and 0.5% iron chelate. Nitrogen concentrations were derived using K_2SO_4 and KNO_3 at different amounts. The six nitrogen treatments had NO_3^- concentrations of 0, 5, 10, 20, 40, and 80 mg l^{-1}. One-hundred milliliter of Hoagland's solution with the desired NO_3^- concentration was applied per pot on February 26 (one month after removal from Pro-Mix); March 8, 15, and 22; April 5 and 26, 1996.

2.3. Vegetative growth

Vegetative growth was measured prior to initial nitrogen treatment and then each week for the duration of the experiment. Crown diameter, number of stolons, total length of stolons, number of flowers, and number of ramets were measured. Weights of leaves, stems, and roots were measured on a representative subsample of each genotype prior to the first N application, on February 9, 1996 and on all plants at the end of the experiment.

All plants were harvested on May 3, 1996. Shoots and roots were separated and weighed. Stolons from each plant were removed and weighed. The ramets were divided into primary (ramets closest to the parent plant), secondary (ramets second from the parent plant), and tertiary (ramets third in line from the parent plant). All ramets beyond tertiary were combined and weighed. Statistical analysis of ramet response to treatment was not different whether ramets were pooled or kept separate by class. Therefore, results are presented for ramets pooled for each strawberry plant. Xylem diameter of each stolon was measured by cutting a cross-section of the stolon and viewing it with an ocular micrometer and a dissecting microscope. Shoots and ramets were ground and nitrogen concentration was measured with a Nitrogen Determinator (Model FP-428, Leco, St. Joseph, MI). Total nitrogen was calculated by multiplying nitrogen concentration by dry weight.

2.4. Layout and design of the experiment

The experiment was completely randomized and a two-way analysis of variance was used to determine genotype and nitrogen effects and their interaction. Each nitrogen treatment was replicated at least three times for all genotypes. Linear regressions were used to characterize the effects of nitrogen treatments within particular genotypes when interactions occurred between genotype and nitrogen treatment. Mean separations of genotype effects were also performed using Fisher's Protected LSD Test.

3. Results

3.1. Genotype

Genotypes R07, DNT, and I19 exhibited a guerrilla type morphology with elongated stolons and widely dispersed ramets. Genotype R07 was most ''guerrilla-like'' with fewest stolons, branch crowns, and ramets (Table 1). Also, Genotype R07 had the longest stolons and internode lengths (Table 2). The dry weights and total N were similar among Genotypes R07, DNT, and I19 but more N was partitioned to parent plants in Genotypes R07 than in Genotypes DNT and I19 (Table 3).

Genotypes DNT and I19 had more moderate guerrilla characteristics than Genotype R07. Although significant genotype×nitrogen interactions occurred, Genotypes DNT and I19 consistently had more stolons, branch crowns, and ramets (approximately 4, 2, and 17, respectively) than Genotype R07 (approximately 3, 1, and 8, respectively) (Table 1). Average stolon length was less in Genotypes DNT and I19 than Genotype R07 (Table 2). Stolon diameters

Table 1
Number of crowns, ramets, and stolons of strawberry genotypes grown with different nitrogen treatments

Genotype[a]	d.f.	Branch crowns (No.)	Ramets (No.)	Stolon (No.)	Stolon diameter (mm)		Ramet density (No./100 cm)
					Stem	Xylem	
R07		1.1	8 c	2.7	2.1 a	1.3 a	3.1
DNT		2.4	17 b	3.9	1.9 b	1.1 b	5.1
Q18		3.6	24 a	8.7	1.6 d	0.8 d	10.3
I19		2.2	16 b	4.2	1.7 c	0.9 c	4.3
Source		$P > F$					
Genotype (G)	3	0.01	0.01	0.01	0.01	0.01	0.01
Nitrogen (N)	5	0.24	0.03	0.01	0.13	0.10	0.02
G×N	15	0.02	0.28	0.01	0.83	0.30	0.01
	127						

[a] ANOVA demonstrated significant effects of genotype or nitrogen treatment. For genotype main effect, means within a column followed by the same letter did not differ at $P \leq 0.05$. Mean separations were not presented where main effects were not significant or where interactions were significant.

differed among genotypes from greatest to least: R07, DNT, I19, and Q18 (Table 1).

Genotype Q18 exhibited characteristics that differed from the other genotypes and was similar to the phalanx morphology. Genotype Q18 had short, bunched stolons. Genotype Q18 had the greatest number of stolons,

Table 2
Crown and stolon size of strawberry genotypes grown with different nitrogen treatments

Genotype[a]	d.f.	Crown diameter (cm)	Stolon length measurements (cm)		
			Average	Total	Internode
R07		13.7 a	105 a	275 b	34 a
DNT		11.5 b	91 b	332 a	20 c
Q18		7.9 c	29 c	249 b	10 d
I19		11.2 b	92 b	376 a	24 b
Source		$P > F$			
Genotype (G)	3	0.01	0.01	0.01	0.01
Nitrogen (N)	5	0.01	0.01	0.12	0.44
G×N	15	0.62	0.14	0.60	0.68
	127				

[a] ANOVA demonstrated significant effects of genotype or nitrogen treatment. Interactions were not significant. For genotype main effect, means within a column followed by the same letter did not differ at $P \leq 0.05$.

Table 3
Nitrogen content and dry weights of strawberry genotypes grown with different nitrogen treatments

Genotype[a]	d.f.	Nitrogen content			Dry weight		
		Parent (% distribution)	Ramet (% distribution)	Total (mg)	Parent (% distribution)	Ramet (% distribution)	Total (g)
R07		47.3 a	52.6 b	10.6 a	37.6 b	62.4 a	11.2 a
DNT		37.7 b	62.3 a	10.0 a	40.4 b	59.6 a	11.4 a
Q18		50.8 a	49.2 b	7.7 b	53.7 a	46.3 b	5.7 b
I19		40.7 b	59.3 a	10.2 a	42.6 b	57.4 a	10.8 a
Source		*P > F*					
Genotype (G)	3	0.01	0.03	0.01	0.01	0.01	0.01
Nitrogen (N)	5	0.10	0.10	0.01	0.10	0.10	0.91
G×N	15	0.16	0.16	0.26	0.46	0.46	0.84
	127						

[a] ANOVA demonstrated significant effects of genotype or nitrogen treatment. Interactions were not significant. For genotype main effect, means within a column followed by the same letter did not differ at $P \leq 0.05$. Mean separations were not presented where main effects were not significant.

branch crowns, and ramets and the shortest stolons (Tables 1 and 2). Unlike Genotypes DNT and I19, more N was partitioned to the parent plant than to the ramets (Table 3).

3.2. Nitrogen treatment

With increasing N the number of stolons and ramets increased but the number of branch crowns did not change (Table 1). An interaction was observed between Genotype and N for number of stolons. Genotype R07 was least responsive to increasing N (Fig. 1). Genotype Q18 contained the lowest N (Table 3) and was most responsive to increasing N (Fig. 1). Genotype I19 was intermediate between Genotypes R07 and Q18 in responding to increasing N. Genotype I19 partitioned more N into ramets than Genotypes Q18 and R07.

The total stolon length was not affected by N while number of stolons was greatest at the highest N (Tables 1 and 2, Fig. 1). However, differences in genotype response to N precludes the generalization that all strawberry plants will exhibit a foraging response. The number of stolons increased most for Genotype Q18 with increased N (Fig. 1). As a result the number of ramets per 100 cm stolon length increased with increased N only for Genotype Q18 (Fig. 2). Consequently, the genotype with the phalanx morphology responded to increased N by greater proliferation of ramets near the parent plant — a foraging response.

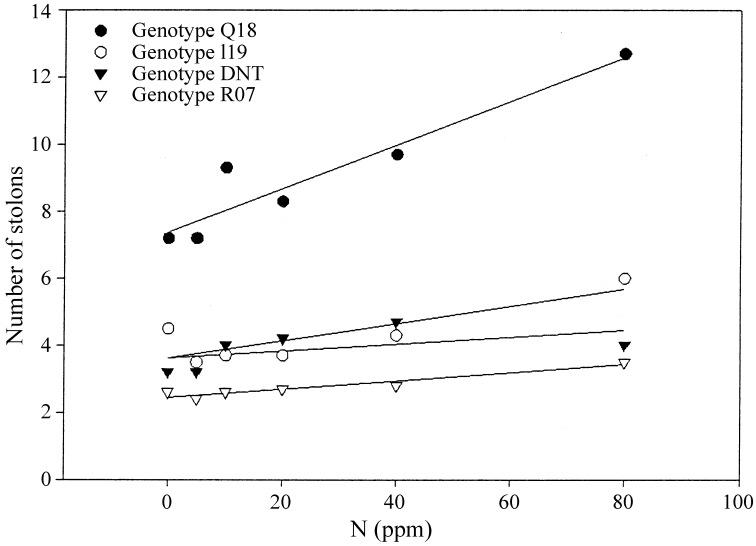

Fig. 1. Number of stolons of four genotypes of *F. chiloensis* L. grown with different concentrations of nitrogen. Average number of stolons for each genotype were plotted at each N concentration. Number of stolons for regression equations for Genotypes Q18, I19, DNT, and R07 were: $7.5 + 0.064\,\mathrm{N}$ $(r^2 = 0.41)$; $14.5 + 0.072\,\mathrm{N}$ $(r^2 = 0.24)$; $16.5 + 0.022\,\mathrm{N}$ $(r^2 = 0.01)$; and $2.5 + 0.012\,\mathrm{N}$ $(r^2 = 0.22)$, respectively. Regression slopes were significantly different from zero at the $P \leq 0.05$ level except for Genotype DNT.

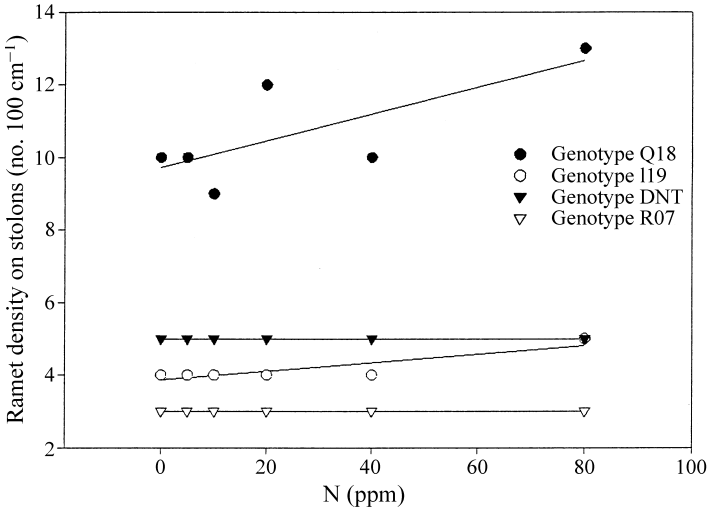

Fig. 2. Ramet density of four genotypes of *F. chiloensis* L. grown with different concentrations of nitrogen. Average ramet density for each genotype were plotted at each N concentration. Number of ramets per 100 cm of stolon of regression equations for Genotypes Q18, I19, DNT, and R07 were: $9.6 + 0.504\,\mathrm{N}$ $(r^2 = 0.14)$; $4.1 + 0.009\,\mathrm{N}$ $(r^2 = 0.19)$; $5.2 - 0.001\,\mathrm{N}$ $(r^2 = 0.002)$; and $3.0 + 0.002\,\mathrm{N}$ $(r^2 = 0.007)$, respectively. The regression for Genotype Q18 was the only relationship with a slope significantly different from zero at the $P \leq 0.05$ level.

4. Discussion

In some clonal plants, stolon length decreased but number of stolons and ramets increased with increased soil fertility (Silberbush and Lips, 1988; Moon et al., 1990). The opposite morphological response has been observed with decreased soil fertility. These morphological traits have been termed a foraging response (Cain, 1994). In the current study, all strawberry genotypes had small, yet significant foraging responses. Number of ramets increased and stolon length decreased at higher N. Other studies found no foraging response. Harnett (1993) observed no increase in ramets with *Panicum virgatum* L. following N treatment and Gray and Call (1993) found decreased plant number of mockstrawberry (*Duchesnea indica* (Andrews) Focke) with fertilization. Cain (1994) determined that stolon lengths decreased in favorable habitats because stolon length was partially dependent on the direction of stolon growth. In other words, shoot architecture of a given plant species may inherently lend itself toward increased branching in favorable environments as a genetic, rather than a phenotypic response (Cain et al., 1991). Foraging response apparently differs among stoloniferous plants.

Number of stolons increased with increasing N in genotypes R07, Q18, and I19. This may indicate support of the foraging response concept, although most authors conclude that increased branching, rather than the number of stolons was more indicative of foraging response. Increased branching in stolons has also been refuted as an indication of foraging response. Cain (1994) indicated that lateral branching may simply result from a plant growing well in a favorable environment and not from a growth response to exploit nutrients. Foraging response theory aside, Alpert (1991) found that stoloniferous plants given high N ($50\ mg\ l^{-1}$) produced more stolons than plants with no N amendments. This concurs with this study for three of the four genotypes investigated. It may be significant that foraging response experiments were often performed by placing mixed genotypes of a single species in a controlled, heterogeneous environment where ramets were allowed to root. The current experiment was unique in that foraging response to N was evaluated among genetically uniform individuals.

Foraging response includes the proliferation of absorptive organs where the plant encounters resource-enriched spaces. One could postulate the physiological underpinning as a balance between stimulated meristem development and restriction of growth beyond an enriched zone. Plant roots can proliferate when they encounter soil enriched in nitrogen (Atkinson, 1974; Daw et al., 1999; Zhang and Forde, 1998) and cytokinin produced from growing root tips exerts control over shoot growth (Richards and Rowe, 1977). It is possible that higher cytokinin resulting from root growth in areas of greater fertility could stimulate growth of new stolons. Exogenous cytokinin and gibberellin can stimulate the number of new stolons in strawberry (Braun and Kender, 1985). However, under conditions

of generally low fertility, as in this experiment, low carbon assimilation may limit growth (Chapin, 1980), resulting in plants with numerous but short stolons. Thus, foraging response of Genotype Q18 in this experiment may be the result of hormone stimulation of stolon meristem activity coupled with inadequate energy to support extensive growth. Morphological plasticity would be necessary for foraging response morphology to occur. Plasticity of stolon growth due to moisture stress has been demonstrated with some genotypes used in this experiment (VanDerZanden and Cameron, 1996).

5. Conclusion

Foraging response among genotypes I19, DNT, R07, and Q18 was not obvious in this experiment, supporting the work of Harnett (1993) and Gray and Call (1993). However, in limited cases, specific to genotype, evidence of foraging response was found. Genotypes I19, R07, and Q18 were responsive to N by increasing number of stolons. Genotype Q18 contained small plants with a phalanx morphology and increased N accentuated the phalanx habit by stimulating more stolons and increasing ramet density. Similar vegetative growth may have occurred with genotypes of the guerrilla morphology if higher N concentrations were studied. In this experiment, foraging response was not a consistent plant response to N variation but foraging response appeared to vary with genotype. Previous experiments have used species which have a branching morphology and may be more likely to exhibit a foraging response (e.g. Birch and Hutchings, 1994). It is also possible that local ecotypes may differ in branching patterns, foraging response, and resource sharing among ramets (Alpert, 1999). The *F. chiloensis* used in this study exhibited a morphology that did not have a branching habit which may partly explain the lack of a strong foraging response. Nevertheless, foraging responses were found to vary among genotypes of *F. chiloensis* with the greatest foraging response associated with strawberry plants of a phalanx growth habit.

References

Alpert, P., 1991. Nitrogen sharing among ramets increases clonal growth in *Fragaria chiloensis*. Ecology 72, 69–80.

Alpert, P., 1999. Clonal integration in *Fragaria chiloensis* differs between populations: ramets from grassland are selfish. Oecologia 120, 69–76.

Atkinson, D., 1974. Some observations on the distribution of root activity in apple trees. Plant and Soil 40, 333–342.

Birch, C.P.D., Hutchings, M.J., 1994. Exploitation of patchily distributed soil resources by the clonal herb *Glecoma hederacea*. J. Ecol. 82, 653–664.

Bish, E.B., Cantliffe, D.J., Hockmuth, C.J., Chandler, C.K., 1997. Development of containerized strawberry transplants for Florida's winter production system. Acta Hort. 439, 461–468.

Braun, J.W., Kender, W.J., 1985. Correlative bud inhibition and growth habit of the strawberry as influenced by application of gibberellic acid, cytokinin, and chilling during short daylength. J. Am. Soc. Hort. Sci. 110, 28–34.

Cain, M.L., 1994. Consequences of foraging in clonal plant species. Ecology 75, 933–944.

Cain, M.L., Carson, W.P., Root, R.B., 1991. Long term suppression of insect herbivores increases the production and growth of *Solidago altissima* rhizomes. Oecologia 88, 251–257.

Chapin, F.S., 1980. The mineral nutrition of wild plants. Ann. Rev. Ecol. Syst. 11, 233–260.

Daw, T., Tworkoski, T.J., Glenn, D.M., 1999. Root restriction and fertilizer effects on young peach trees. HortScience 34, 494 (Abstract).

DeKroon, H., Hutchings, M.J., 1995. Morphological plasticity in clonal plants: the foraging concept reconsidered. J. Ecol. 88, 143–152.

Gleason, H.A., Cronquist, A., 1991. Manual of Vascular Plants of Northeastern United States and Adjacent Canada. New York Botanical Garden, Bronx, NY.

Gray, E., Call, N.M., 1993. Fertilization and mowing on persistence of Indian mockstrawberry (*Duchesnea indica*) and common blue violet (*Viola papilionaceae*) in tall fescue (*Festuca arundinacea*) lawn. Weed Sci. 41, 548–550.

Hamann, K.K., Poling, E.B., 1997. The influence of runner order, night temperature and chilling cycles on the earliness of 'Selva' plug plant fruit production. Acta Hort. 439, 597–603.

Hancock, J.F., Bringhurst, R.S., 1979. Ecological differentiation in perennial octoploid species of *Fragaria*. Am. J. Bot. 66, 367–375.

Harnett, D.C., 1993. Regulation of clonal growth and dynamics of *Panicum virgatum* (Poaceae) in a tallgrass prairie: effects of neighbor removal and nutrient addition. Am. J. Bot. 80, 114–120.

Hoagland, D.R., Arnon, D.I., 1950. The water culture method of growing plants without soil. California Agricultural Experiment Station Circular No. 347.

Kelly, C.K., 1994. On the economics of plant growth: stolon length and ramet initiation in the parasitic clonal plant *Cuscuta europaea*. Evol. Ecol. 8, 459–470.

Lovett Doust, L., 1981. Interclonal variation and competition in *Ranunculus repens*. New Phytol. 89, 495–502.

Moon Jr., J.W., Bailey, D.A., Fallahi, E., Jensen, R.G., Zhu, G., 1990. Effect of nitrogen application on growth and photosynthetic nitrogen use efficiency in two ecotypes of wild strawberry, *Fragaria chiloensis*. Physiol. Plantarum 80, 612–618.

Richards, D., Rowe, R.N., 1977. Root–shoot interactions in peach: the function of the root. Ann. Bot. 41, 1211–1216.

Silander, J.A., 1985. Microevolution in clonal plants. In: Jackson, J.B.C., Buss, L.W., Cook, R.E. (Eds.), Population Biology and Evolution of Clonal Organisms. Yale University Press, New Haven, CT, pp. 107–152.

Silberbush, M., Lips, S.H., 1988. Nitrogen concentration, ammonium/nitrate ratio and NaCl interaction in vegetative and reproductive growth of peanuts. Physiol. Plantarum 74, 493–498.

Takeda, F., 1999. Soilless strawberry production. Adv. Strawberry Res. 19, 1–8.

Turkington, R., Klein, E., 1993. Influence of neighbors on node production, stolon length, and branching of *Trifolium repens* transplants in a pasture. Can. J. Bot. 71, 1266–1296.

VanDerZanden, A.M., Cameron, J.S., 1996. Effect of water deficit stress on 11 native *Fragaria chiloensis* clones selected as ornamental groundcovers. Sci. Hort. 66, 241–253.

Wijesinghe, D.K., Handel, S.N., 1994. Advantages of clonal growth in heterogeneous habitats: an experiment with *Potentilla simplex*. J. Ecol. 82, 495–502.

Zhang, H., Forde, B.G., 1998. An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. Science 279, 407–409.