The Ecological Significance of Clonal Growth in the Understory Tree, Pawpaw (*Asimina triloba*)

Naomi Hosaka[1, 2, 3,*], Sara Gómez[2], Naoki Kachi[1], Josef F. Stuefer[2], and Dennis F. Whigham[3]

Abstract - We evaluated three possible functions of clonal growth related to genet persistence in the root-suckering understory tree pawpaw, *Asimina triloba* (L.) Dunal: (1) risk spreading through multiplication of stems, (2) enhanced establishment and survival of new stems, and (3) horizontal expansion growth of patches. The number, diameter growth, and spatial distribution of annual stem recruits were examined over three years in a natural population of pawpaw. The rate of stem recruitment was consistently higher than stem mortality. We found no difference in stem turnover rate for patches of different size, indicating that stem production is more than high enough to avoid patch extinction. Although newly formed stems were considerably smaller than previously established stems, they grew and survived as well as established stems. We found no evidence for clonal growth contributing to extensive horizontal expansion of patches. Our results suggest that ensuring survivorship of new stems is the main ecological role of clonal growth in pawpaw.

Introduction

Clonal plants produce genetically identical offspring (ramets) that have the potential to become independent of the parent plant. Genetic individuals of clonal plants (genets) therefore often consist of several to many ramets forming spatially distinctive patches in their natural habitats.

The persistence of genets depends on the production, survival, and growth of ramets. Studies on herbaceous species have identified three main ways in which clonal growth can contribute to genet persistence: (1) ramet production can strongly reduce the risk of genet mortality that results from small-scale stochastic disturbances (Eriksson and Jerling 1990), (2) physiological integration promotes the successful establishment of parentally subsidized ramets under harsh environmental conditions (Pitelka and Ashmun 1985), and (3) horizontal expansion of genets by the placement of new ramets may improve the access to heterogeneously distributed resources (De Kroon and Hutchings 1995). Although these functions are not mutually exclusive, their relative importance is likely to differ according to environmental setting and life history characteristics of species.

[1]Tokyo Metropolitan University, Minami-Osawa 1-1, Hachioji, Tokyo 192-0397, Japan. [2]Radbond University Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands. [3]Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD 21037. *Corresponding author - nhosaka@comp.metro-u.ac.jp.

A considerable number of woody plants show spontaneous clonal growth by layering, rhizome production, or root suckering (Del Tredici 2001). Among woody clonal plant species, the shade-intolerant canopy tree, Quaking aspen (*Populus tremuloides* Michx.), is the most widely studied example (Frey et al. 2003, and references therein). In temperate deciduous forests, more understory trees than canopy trees show spontaneous clonal growth (Jenik 1994, Peterson and Jones 1997). Nevertheless, the ecological implications of clonal growth have never been studied explicitly for this group of plants. This paper aims at assessing three possible contributions of clonal growth to genet persistence in understory trees with respect to environmental factors that prevail in their natural habitats.

Risk spreading

Small trees in the forest understory are often damaged by falling debris such as dead trunks or broken branches (Korning and Balslev 1994, Zimmerman et al. 1994). Such frequent small-scale disturbance can randomly kill individual ramets of clonal understory trees (De Steven 1989). The chance that an entire genet will die from such small-scale events equals the product of the probabilities of individual ramets dying, and therefore decreases with an increasing number of ramets per genet (Eriksson and Jerling 1990). Reducing the risk of genet mortality by new ramet production should be more important for genets that consist of a small number of ramets, implying that ramet recruitment rates should be higher in small patches than in large patches.

Subsidized offspring development

Low light availabilities are likely to limit growth and survival of established trees as well as seedlings and saplings in understory environments. Seedlings are usually the most vulnerable stage in the life cycle of trees (Harcombe 1987), and can have high rates of mortality. Clonally produced offspring, on the other hand, can be supported with essential resources by parent plants via stem or root connections, thereby compensating for local resource deficiency (Debyle 1964, Jones and Raynal 1986, Zhang et al. 2002). Vigorous growth of new ramets under shaded conditions can hence be considered as a facilitator of genet persistence and may represent a key advantage of clonal versus non-clonal plants.

Horizontal expansion

In addition to the average light availability, strong spatial heterogeneity in light conditions is a prominent feature of most understory habitats (Nicotra et al. 1999). In temperate deciduous forests, much of the environmental variation is associated with canopy gaps that range from openings created by snapping of branches to gaps created by the

death of one or more canopy trees (Nakashizuka and Iida 1995, Runkle 1990). Horizontal expansion of genets, such as a radial expansion of patches by clonal growth, may increase the likelihood of encountering favorable micro-habitats. Sending out ramets and occupying space beyond the current patch borders have the potential to significantly improve the extraction of scarce resources from the environment, thereby contributing to genet performance and persistence.

More specifically, we will ask the following three questions: (1) Do small patches have a higher ramet production rate than large patches? (2) Do newly produced ramets grow and survive as well as previously established ramets? and (3) Is the density of new ramets higher at the edge than inside of patches? These questions will be answered by presenting results of demographic censuses of the clonal understory tree pawpaw, *Asimina triloba* (L.) Dunal. We will mainly focus on three characteristics of newly produced ramets (numbers, vigor, and spatial distribution) of our model species.

Methods

Study species and site

Pawpaw is native to eastern North America, where it grows in the understory of temperate deciduous forests from northern Florida to southern Ontario (Kral 1960). Pawpaws produce ramets by root suckering. Although individual ramets can become autonomous and grow to a height of more than 10 m, root connections between ramets are likely to remain intact for several, possible many years (Karizumi 1979). Preliminary studies have shown that severing root connections between neighboring pawpaw ramets reduces growth of daughter ramets in terms of height growth and leaf size (unpubl. data, J.F. Stuefer), suggesting that root connections remain functional and that physiological integration is important for the growth and development of ramets.

This study was conducted in a temperate deciduous forest at the Smithsonian Environmental Research Center (SERC), MD (38°53'N, 76°33'W). The forest canopy reaches a height of 25–35 m and is dominated by deciduous broad-leaved trees such as tulip poplar (*Liriodendron tulipifera* L.). (Parker et al. 1989). In June 1999, we individually tagged and numbered all stems of pawpaw in a 7-hectare plot within a forested area at the SERC, locally known as Western Triangle. The understory of the study plot was heavily shaded except for gaps caused by single tree falls.

Data collection

A demographic census of pawpaw was conducted annually from 1999 to 2002 between June and September, after most of the annual growth and recruitment had been completed. In 1999 we found a total of

34 patches of various sizes that were at least 3 m apart from each other. All stems, including newly established stems in any of the 34 patches, were tagged and identified during the subsequent three years. Throughout this study we will use the term *stem* to refer both to clonally and sexually produced plants. The origin of new stems (ramet or seedling) in the patches is basically unknown, but more than 90% of recruits are usually of clonal origin as asserted by inspection of root connections by excavating pawpaw patches in an area adjacent to the study plot (unpubl. data, N. Hosaka). Most stems consisted of a single trunk and a few lateral branches, which is typical for pawpaw stems in heavily shaded forest understory environments. At each annual census we recorded the basal stem diameter at 3 cm above the ground for each tagged stem. A stem was considered dead if it had no leaves or if it was missing.

In September 2002, the four largest patches in the study plot were chosen to investigate the spatial pattern of new stem production. Two of these patches consisted of approximately 140 stems each, while each of the other two patches had more than 500 stems. We divided each patch into quarters by two perpendicular axes intersecting at the center of the patch. Each of the quarters was then subdivided into three sectors with equal areas. The sectors were established along the axes extending from the center to the periphery of the patch (Fig. 1). We counted the number of stems in each of these sectors.

Data analysis

A reduced major axis (RMA) regression (Sokal and Rohlf 1995) was used to examine stem natality and stem mortality in relation to patch size. The RMA regression procedure is appropriate if both the dependent and the independent variables in the regression model cannot be measured without error. Stem natality and mortality were defined as the sum of new stems and the sum of dead stems over the whole study period, respectively. We regressed natality and mortality on the initial patch size, which we defined as the total number of stems present in each patch at the first census. All values were increased by 0.1 and plotted in logarithmic scale on both axes. The 95% confidence intervals
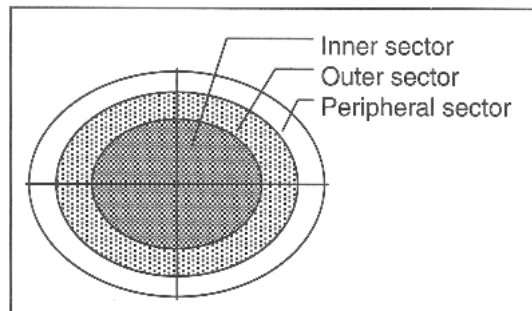

Figure 1. Schematic representation of a large pawpaw patch, which was sub-divided into quarters and sectors (inner, outer, and peripheral) for measuring stem density. The three sectors within each quarter are of the same size.

of the regression slopes were used to evaluate whether regression coefficients were statistically different from each other. In this analysis, a slope of 0 indicates that the stem natality or mortality is independent of initial patch size. A slope of 1 means that the stem natality or mortality increases with the initial patch size, but that there are no changes in their percentages associated with increasing patch size.

The survivorship and growth of newly established stems were compared with those of previously established stems. Two cohorts of new stems, namely the ones established in 2000 and 2001, were analyzed separately. We used G-tests to analyze stem survivorship, testing the null-hypothesis that the chance of individual stems dying was independent from whether they were new or old stems. Differences in stem diameter and absolute stem diameter growth between new and old stems were analyzed using paired t-tests. Since stems within patches cannot be considered independent we calculated mean values of stem diameter and diameter increment for new and old stems within each patch and analyzed the difference between those means. Patches without new stems were excluded from the data sets.

To evaluate whether new stem production varied with spatial position within patches, we calculated the density of new stems (i.e., established after 1999) and older stems in each of the three sectors (inner, outer, and peripheral sector). To improve accuracy, stem density was measured within each quarter, but mean densities within sectors were used in the analysis. The effects of stem status (new or old) and spatial position (inner, outer, and peripheral sectors) on new stem production were tested by means of a repeated-measures ANOVA, using status as a between-subject factor. Since the three levels of position (inner, outer, and peripheral) are likely to show spatial autocorrelation, position was treated as a within-subject factor. This is formally identical to a split-plot analysis considering the segments split plots (Von Ende 2001).

Results

Stem natality and stem mortality

At the first census in 1999, the initial number of stems per patch ranged from 1 to more than 500. All patches increased in number of stems over the study period, except for two very small patches that decreased in stem numbers due to the death of a single stem. Stem natality was consistently higher than stem mortality (Fig. 2). The slopes of the regression lines for natality and mortality were not significantly different from 1 (95% confidence intervals of the slopes for new and dead stems were 0.982–1.489 and 0.999–1.584, respectively). This result indicates that small patches did not differ from large patches in their relative production rate and death rate of stems. In other words, the rate of stem turnover was constant over a large trajectory of patch sizes.

Growth and survivorship

The basal diameter of new stems was significantly smaller than the diameter of previously established stems (Fig. 3; Table 1). Nevertheless, both new stem cohorts established in 2000 and 2001 grew as vigorously as old stems in terms of absolute diameter increment (Table 1). New stems survived as well as older stems. Neither of the new stem cohorts established in 2000 and 2001 showed significant differences in survivorship from old stems during their first year of life (Table 2).

Stem density

New stems were found throughout the patches, and their density tended to be high in the area close to the center (Fig. 4). The effect of spatial position within patches (inner, outer, and peripheral sectors) on
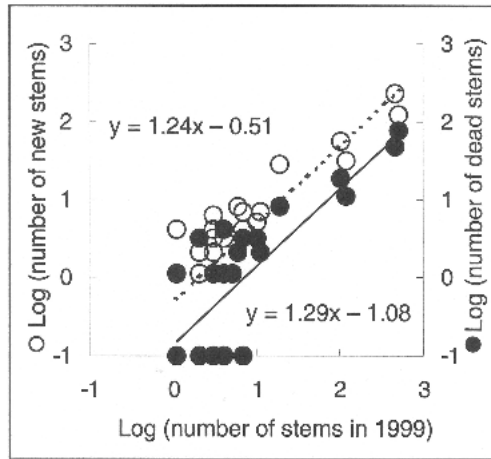

Figure 2. Relationship between the initial number of stems per patch (x-axis) and natality (dashed line) and mortality (solid line). Open and closed circles represent the number of new and dead stems, respectively, summed over three years for each patch. All values were increased by 0.1 to allow for log-transformation.
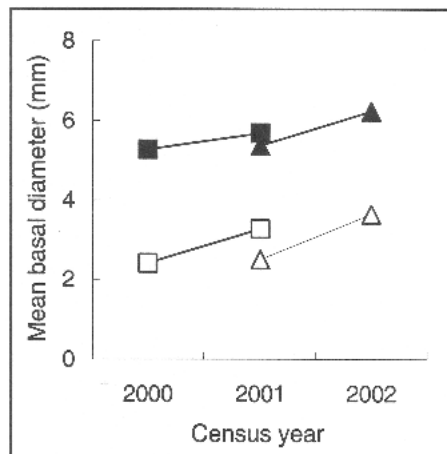
$y = 1.24x - 0.51$

$y = 1.29x - 1.08$

Figure 3. Mean basal diameter of new (open) and old (closed) pawpaw stems. Different symbols are used for the two cohorts established in two successive years (2000 and 2001). The results of the statistical analysis of these data sets are shown in Table 1.

Table 1. Stem size and annual growth in basal stem diameter. The data are based on mean values (calculated for each patch) for new and old stems. Means ± SE are reported. P-values are based on paired t-tests.

Variable	n	New	Old	Difference	P
Basal diameter (mm)					
2000	17	2.52 ± 0.22	4.60 ± 0.30	-2.08 ± 0.31	< .0001
2001	20	2.56 ± 0.15	5.47 ± 0.40	-2.91 ± 0.36	< .0001
Absolute diameter growth (mm)					
2000–2001	17	0.75 ± 0.16	0.40 ± 0.15	0.26 ± 0.21	0.2399
2001–2002	20	1.20 ± 0.12	1.17 ± 0.15	0.03 ± 0.14	0.8138

Table 2. Results of a G-test analyzing differences in survivorship between new and old stems during 2000–2001 and 2001–2002.

Period	Number of new stems	Total number of stems	Expected proportion	Observed proportion	G
2000–2001	193	1457	0.132	0.125	0.7382[ns]
2001–2002	175	1564	0.112	0.113	0.0059[ns]

[ns] $P > 0.05$

Table 3. Results of repeated-measures ANOVA of new stem density in the four largest pawpaw patches of the study plot.

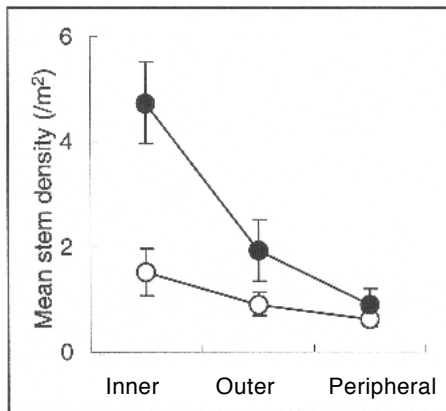| Source	df	MS	F	P
Between-subject-effects				
Status	1	13.7048	12.50	0.0123
Error (between)	6	1.0965		
Within-subject-effects				
Position	2	12.0143	19.74	0.0007
Position x status	2	4.7065	5.14	0.0170
Error (within)	12	0.7576		

Figure 4. Mean ± SE densities (stems/m^2) of new (open) and old (closed) stems in the four largest pawpaw patches in the study plot. The analyses of these data sets are given in Table 3.

stem density was significant in the repeated–measures ANOVA (Table 3). This tendency was even more pronounced for old stems (Fig. 4) as indicated by a significant interaction term between stem status (old and new) and spatial position within patches (Table 3). The effect of stem status on density was also significant due to a high density of old stems in the inner sectors (Fig. 4; Table 3). These effects are most likely due to a local accumulation of stem cohorts over years.

Discussion

The data presented in this paper provide insight into possible ecological functions of clonal growth for the persistence of a clonal understory tree in its natural environment. While these functions are reasonably well understood for herbaceous species from a variety of habitats, there is still little insight into the ecological role of clonality in trees. Our study supports the view that predictions and conclusions about the significance of clonal growth cannot be easily extrapolated from herbaceous species to clonal understory trees. Risk spreading and horizontal habitat exploration seem not to be primary functions of clonal growth in our model species. Clonal growth appears to contribute mainly to high survivorship of individual ramets by ensured ramet establishment in low-light environments.

Our demographic study has shown that stem natality was consistently higher than stem mortality across a wide range of patch sizes, indicating that pawpaw patches keep growing in stem numbers regardless of clone age. Net stem production was higher than necessary to avoid patch extinction caused by frequent disturbances of falling debris from the forest canopy. Since small patches did not show an increased natality rate in comparison to larger patches, our results do not support the idea of risk spreading being the main ecological function of clonal growth in this system.

In spite of high rates of stem damage by falling tree branches (unpubl. data, N. Hosaka and J.F. Stuefer) no patch extinction was observed over the three-year census period. Even the smallest patches consisting of only one or a few stems did not suffer from extinction. This seeming contradiction may be explained by a high re-sprouting potential of damaged pawpaw stems. Many angiosperms and a few gymnosperm trees species sprout as an induced response to injury (Bond and Midgley 2003, Del Tredici 2001). Clonal trees are no exceptions to this rule, but to date we do not have a good understanding of the interplay between spontaneous clonal growth and damage-induced re-sprouting in trees. For our species, we propose that a high re-sprouting ability of damaged stems, rather than the induction of clonal growth and new stem production, may compensate for the

partial loss of stems through physical disturbance in the forest understory. This hypothesis could be tested in field experiments that simulate natural disturbance.

The high survivorship of new pawpaw stems supports our second idea of improved genet persistence through a enhanced establishment of ramets. Similar results have been obtained for other clonal understory trees (Kuuseoks et al. 2001, Tappeiner and McDonald 1984, Whitney 1986) and for some shade-tolerant canopy trees (Jones and Raynal 1988). In contrast, low survivorship of new stems was reported for clonal woody species that inhabit forest edges or early seral stages of forests systems (Huenneke 1987, Luken 1990, Nantel and Gagnon 1999, Tappeiner et al. 2001). New stems of these species appear to be suppressed by neighboring old stems. In our study, new stems grew as vigorously as old stems, which is probably due to physiological support from parent plants via root connections. Successful establishment and high survivorship of newly produced stems may represent a cost-saving strategy that enables pawpaw to maintain its patches over long periods of time at low structural costs. Safe investments into successful offspring stems seem an essential prerequisite for long-term persistence of pawpaw in the lower layers of a dense forest understory.

The density of newly produced stems was higher in the interior of the patches as compared to the periphery. Hence, our data did not support the notion of horizontal genet expansion being a major function of clonal growth in pawpaw. This result contrasts with reports for many herbaceous species (Adachi et al. 1996, Wikberg and Svensson 2003) and clonal pioneer trees whose ramets occur mainly on the periphery of patches (Debyle 1964, Gilbert 1966, Mishio and Kawakubo 1998). Our results suggest that the spatial pattern of new stem establishment of pawpaw is not efficient for horizontal expansion and habitat exploration by means of radial patch growth. Nevertheless, it is likely to facilitate local persistence over potentially prolonged periods of time, thereby increasing the chance of encountering changes in light condition, which may occur from time to time due to larger scale canopy damage. Exploiting long-term temporal variation in light availability, rather than exploring spatial variation, may be a more feasible strategy for a species like pawpaw. The high within-branch variation of leaf characteristics associated with temporal variation in light condition reported for pawpaw (Young and Yavitt 1987) is consistent with this idea. However, a direct comparison of performance and clonal behavior of pawpaw between high light and low light environments would be necessary to further clarify this point.

In conclusion, this study evaluated different ecological aspects of spontaneous clonal growth in an understory tree species from temperate deciduous forests. Our results suggest that ensuring survivorship of

individual stems is the major function of clonal growth in pawpaw, and possibly also in other ecologically similar understory tree species. We propose that pawpaw adopts a sit-and-wait strategy which allows the species to exploit infrequent and unpredictable changes in local light conditions such as caused by larger tree falls.

Acknowledgments

Literature Cited

Adachi, N., I. Terashima, and M. Takahashi. 1996. Central die-back of mono-clonal stands of *Reynoutria japonica* in an early stage of primary succession on Mount Fuji. Annals of Botany 77:477–486.

Bond, W.J., and J.J. Midgley. 2003. The evolutionary ecology of sprouting in woody plants. International Journal of Plant Sciences 164:S103-S114.

Debyle, N.V. 1964. Detection of functional intraclonal root connections by tracer and excavation. Forest Science 10:386–396.

De Kroon, H., and M.J. Hutchings. 1995. Morphological plasticity in clonal plants: The foraging concept reconsidered. Journal of Ecology 83:143–152.

Del Tredici, P. 2001. Sprouting in temperate trees: A morphological and eco-logical review. The Botanical Review 67:121–140.

De Steven, D. 1989. Genet and ramet demography of *Oenocarpus mapora* ssp. *mapora*, a clonal palm of Panamanian tropical moist forest. Journal of Ecology 77:579–596.

Eriksson, O., and L. Jerling. 1990. Hierarchical selection and risk spreading in clonal plants. Pp. 79–94, *In* J. van Groenendael and H. de Kroon (Eds.). Clonal Growth in Plants. SPB Academic Publishing, Hague, The Nether-lands. 196 pp.

Frey, B.R., V.J. Lieffers, S.M. Landhausser, P.G. Comeau, and K.J. Greenway. 2003. An analysis of sucker regeneration of trembling aspen. Canadian Journal of Forest Research 33:1169–1179.

Gilbert, E.F. 1966. Structure and development of sumac clones. The American Midland Naturalist 75:432–445.

Harcombe, P.A. 1987. Tree life tables. BioScience 37:557–568.

Huenneke, L.F. 1987. Demography of a clonal shrub, *Alnus incana* ssp. *rugosa* (Betulaceae). The American Midland Naturalist 117:43–55.

Jenik, J. 1994. Clonal growth in woody plants: A review. Folia Geobotanica et Phytotaxonomica 29:291–306.

Jones, R.H., and D.J. Raynal. 1986. Spatial distribution and development of root sprouts in *Fagus grandifolia* (Fagaceae). American Journal of Botany 73:1723–1731.

Jones, R.H., and D.J. Raynal. 1988. Root sprouting in American beech (*Fagus grandifolia*): Effects of root injury, root exposure, and season. Forest Ecology and Management 25:79–90.

Karizumi, N. 1979. Illustrations of Tree Roots. Seibundo Shinkosha Publishing, Tokyo, Japan. 1137 pp.

Korning, J., and H. Balslev. 1994. Growth and mortality of trees in Amazonian tropical rain forest in Ecuador. Journal of Vegetation Science 4:77–86.

Kral, R. 1960. A revision of *Asimina* and *Deeringothamus* (Annonaceae). Brittonia 12:233–277.

Kuuseoks, E., J. Dong, and D. Reed. 2001. Shrub age structure in northern Minnesota aspen stands. Forest Ecology and Management. 149:265–274.

Luken, J.O. 1990. Gradual and episodic changes in the structure of *Rhus typhina* clones. Bulletin of the Torrey Botanical Club 117:221–225.

Mishio, M., and N. Kawakubo. 1998. Ramet production by *Mallotus japonicus*, a common pioneer tree in temperate Japan. Journal of Plant Research 111:459–462.

Nakashizuka, T., and S. Iida. 1995. Composition, dynamics and disturbance regime of temperate deciduous forest in Monsoon Asia. Vegetatio 121:23–30.

Nantel, P., and D. Gagnon. 1999. Variability in the dynamics of northern peripheral versus southern populations of two clonal plant species, *Helianthus divaricatus* and *Rhus aromatica*. Journal of Ecology 87:748–760.

Nicotra, A.B., R.L. Chazdon, and S.V.B. Iriarte. 1999. Spatial heterogeneity of light and woody seedling regeneration in tropical wet forests. Ecology 80:1908–1926.

Parker, G.G., J.P. O'Neill, and D. Higman. 1989. Vertical profile and canopy organization in a mixed deciduous forest. Vegetatio 85:1–11.

Peterson, C.J., and R.H. Jones. 1997. Clonality in woody plants: A review and comparison with clonal herbs. Pp. 263–289, *In* H. de Kroon and J. van Groenendael (Eds.). The Ecology and Evolution of Clonal Plants. Backhuys Publishing, Leiden, The Netherlands. 453 pp.

Pitelka, L.F., and J.W. Ashmun. 1985. Physiology and integration of ramets in clonal plants. Pp. 399–435, *In* J.B.C. Jackson, L.W. Buss, and R.E. Cook (Eds.). Population Biology and Evolution of Clonal Organisms. Yale University Press, New Haven, CT. 530 pp.

Runkle, J.R. 1990. Gap dynamics in an Ohio, USA *Acer-Fagus* forest and speculations on the geography of disturbance. Canadian Journal of Forest Research 20:632–641.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry. W.H. Freeman and Company, New York. NY. 887 pp.

Tappeiner, J.C., II, and P.M. McDonald. 1984. Development of tanoak understories in conifer stands. Canadian Journal of Forest Research 14:271–277.

Tappeiner, J.C., II, J.C. Zasada, D.W. Huffman, and L.M. Ganio. 2001. Salmonberry and salal annual aerial stem production: The maintenance of shrub cover in forest stands. Canadian Journal of Forest Research 31:1629–1638.

Von Ende, C.N. 2001. Repeated-measures analysis: Growth and other time-dependent measures. Pp. 134–157, *In* S.M. Scheiner and J. Gurevitch (Eds.). Design and Analysis of Ecological Experiments. Oxford University Press, New York, NY. 415 pp.

Whitney, G.G. 1986. A demographic analysis of *Rubus idaeus* and *Rubus pubescens*. Canadian Journal of Botany 64:2916–2921.

Wikberg, S., and B.M. Svensson. 2003. Ramet demography in a ring-forming clonal sedge. Journal of Ecology. 91:847–854.

Young, D.R., and J.B. Yavitt. 1987. Differences in leaf structure, chlorophyll, and nutrients for the understory tree *Asimina triloba*. American Journal of Botany 74:1487–1285.

Zhang, C., C. Yang, and M. Dong. 2002. Clonal integration and its ecological significance in *Hedysarum laeve*, a rhizomatous shrub in Mu Us Sandland. Journal of Plant Research 115:113–118.

Zimmerman, J.K., E.M. Everham, R.B. Waide, D.J. Lodge, C.M. Taylor, and N. Brokaw. 1994. Responses of tree species to hurricane winds in subtropical wet forest in Puerto Rico: Implications for tropical tree life histories. Journal of Ecology 82:911–922.