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Why it matters where on a leaf a folivore feeds

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Abstract Because the tip of many dicot leaves matures and ceases expansion well before the base, we predicted that the removal of a given amount of leaf tissue from the base of an expanding leaf would result in greater reductions in final leaf area and overall plant performance than removal of the same amount of tissue from the tip of an expanding leaf or from either the base or tip of mature, fully expanded leaves. We tested this notion by removing a circular 3.9 cm² hole from either the base or tip of rapidly expanding leaves (20–30% expanded, two nodes from the apex) or nearly fully expanded, mature leaves (85–100% expanded, five nodes from the apex) of tobacco (*Nicotiana tabacum*) and measuring the final area of the hole, the final area of the fully expanded damaged leaf, and the number and mass of fruits produced by a plant. A given amount of area removed from the base of an expanding leaf resulted in almost 4 times the amount of visible damage than occurred when the same amount of damage was applied to the tip or base of a mature leaf and over twice the amount of visible damage than occurred on the tip of an expanding leaf. Furthermore, damage to the base of an expanding leaf resulted in nearly a 40% reduction in the final area that the leaf would have achieved without damage and a 35% reduction in the number and mass of fruits produced. These results not only suggest that where on a leaf a folivore feeds has consequences to the ultimate area that a leaf can reach and to overall plant performance, but they also have strong implications for a number of research areas in plant-herbivore interactions. For example, these data show that a lack of consideration of leaf developmental patterns can result in gross overestimates of consumption by folivores and severe under-estimates of the effect of folivory on leaf area display.

Key words Leaf ontogeny · Leaf development
Nicotiana · Optimal defense · Plant-insect interactions

Introduction

Suites of leaf characteristics that may be important to folivorous insects, such as the tissue concentration of nitrogen and various secondary compounds, change dramatically as leaves age and go through their developmental sequence (Coleman 1986). Not surprisingly, many herbivores show differential preference or performance as a function of the developmental stage or age of leaves upon which they feed (e.g., Raupp and Denno 1983; Coleman 1986; Stamp and Bowers 1990). These observations have led to a number of ecological and evolutionary hypotheses regarding the interrelationships between leaf age, leaf quality, the defense of leaves and the tissue choice of herbivorous insects (e.g., McKey 1979; Cates 1981; Raupp and Denno 1983; Aide and Londoño 1989). For example, optimal defense theory predicts that plants can optimize their defense from herbivory by allocating the greatest proportion of resources for defense to young leaves relative to mature leaves (McKey 1979; Mooney and Gulmon 1982), because young leaves have higher concentrations of nitrogen and have the potential to contribute the greatest amount of photosynthate to the plant (e.g., Mooney and Gulmon 1982; Harper 1989). A prediction that can be made from this theory is that damage by herbivores to young leaves should have a more negative impact on a plant than damage to mature leaves. However, tests of this idea have produced equivocal results (e.g., Cranshaw and Radcliffe 1980; Marquis 1992; Mauricio et al. 1993).

One potential reason for the inconsistency of results is that leaf developmental patterns have not been fully incorporated into experimental designs (see Coleman and Jones 1991; Coleman et al. 1992). For example, “young” expanding leaves differ greatly from one another in many aspects of anatomy, biochemistry and physiology because these factors change dramatically as leaves pass through early phases of expansion (e.g., Isebrands and Larson 1973; Coleman 1986; Dickson and Isebrands 1991). Consequently, a “young” leaf that is only 20% expanded has very different anatomical, physiological, and biochemical

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characteristics than a leaf that is 60% expanded (Coleman 1986). It is therefore possible that damage to one of these two “young” leaves will have very different effects on the plant. Mature leaves, on the other hand, irrespective of their relative chronological ages, tend to have quite similar characteristics (Coleman 1986). To complicate matters further, different areas of a leaf (i.e., base and tip) do not mature synchronously (e.g., Isebrands and Larson 1973; Dickson and Isebrands 1991). Damage to the tip of an expanding leaf might therefore have very different effects than damage to the base of that same leaf.

In this paper we show that an understanding of the dynamics of leaf expansion and development can be used to make explicit predictions regarding the impact that a given amount of herbivore feeding on a given leaf, as well as in a specific location within a leaf, will have on plant performance. In many dicots with simple leaves, such as cottonwood (*Populus deltoides*) or tobacco (*Nicotiana tabacum*), leaf expansion proceeds rapidly in a synchronized manner (Avery 1933; Isebrands and Larson 1973; Esau 1977). The maturation of leaf tissues occurs first at the tip of the leaf and proceeds basipetally, whereas maturation of vascular tissue occurs first at the base of the leaf and proceeds acropetally (e.g., Isebrands and Larson 1973). Leaf expansion is therefore completed early in leaf development at the tip of the leaf and much later at the base of the leaf (Esau 1977; Isebrands and Larson 1973; Dickson and Isebrands 1991). For example, in tobacco and cottonwood, maturation of the leaf tip has already occurred or is very close to completion in leaves that are roughly 20–30% of full expansion. However, maturation at the base of the leaf will not occur until leaves are 90–100% expanded, which is often at a position three to five nodes further down the stem (e.g., Avery 1933; Isebrands and Larson 1973).

It is likely, then, that removal of leaf tissue at the base of an expanding leaf (circa 20–30% expanded) by a chewing herbivore would have a strong negative effect on the expansion of that leaf. This would occur because a large proportion of cells that have not completed expanding in size, as well as intercalary meristems responsible for the production of new cells, would be removed. Additionally, damage to the base of an expanding leaf might sever vascular connections responsible for bringing water and carbohydrates into the leaf. Because these materials are needed for the expansion of the remaining cells and the activity of the remaining intercalary meristems, this would result in even greater decreases in leaf size (e.g., Dickson and Isebrands 1991). Decreases in leaf area, in turn, can have significant effects on the total amount of carbon that the leaf can gain through photosynthesis and, ultimately, on overall plant performance (e.g., Harper 1989). Alternatively, since the tip of this same leaf is far closer to maturation, removal of the same amount of leaf area should have a much smaller impact on the final size that leaf would reach at full expansion, and perhaps a smaller effect on plant growth or fitness. Here we test this notion on tobacco plants using simulated herbivory.

Materials and methods

Seeds of tobacco (*Nicotiana tabacum* cultivar LAFC 53) were obtained from Dr. Ian Baldwin, State University of New York - Buffalo. Tobacco was used because its leaf development has been studied extensively, and leaf development in this species is considered to represent a general model for the development of simple leaves in many dicots (Avery 1933; Esau 1977).

Seeds were germinated in a commercial potting soil (Hyponex All-Purpose Potting Soil, Hyponex Corporation, Marysville, Ohio) by placing the seeds on the surface of the moist soil and covering the container with transparent plastic. When the seedlings had produced at least one true leaf (approximately 9 days after planting) they were transplanted into 1 l plastic pots with fresh potting soil in a greenhouse. The greenhouse contained supplemental lighting by sodium-halide lamps so daytime photosynthetic photon flux densities never dropped below 450 $\mu\text{mol}/\text{m}^2$ per s^{-1} . The pots were placed in plastic containers to minimize the loss of nutrients and water from the plant-pot system. Plants were watered every other day, and this provided enough water to prevent any visible signs of wilting. Ten days after transplantation, plants were given 0.40 g fertilizer (20:20:20, NPK) dissolved in 150 ml of distilled water. This dose of fertilizer was repeated again 20 days later. These doses of fertilizer allowed for vigorous growth of plants and a constant rate of leaf production as measured via the plastochron index (see Lamoreaux et al. 1978; approximately 0.45 leaves/day) for the complete course of vegetative growth. Leaves consistently reached 85–95% full expansion when they reached the fifth node from the apex. Leaves at the second node were consistently 20–30% the size of fully expanded leaves. Leaves at these two developmental stages were used in the experiment.

Damage treatments were applied 25 days after transplantation using a cork borer to remove a circular 3.9 cm^2 hole from the leaf. Plants had between eight and ten leaves at this time. There were five groups of plants and five replicate plants within each group. The treatments were: (1) hole removed at the tip of a leaf two nodes down the stem from the most newly unfolded leaf (i.e., leaves at 20–30% full expansion); (2) hole removed at the base of the leaf at this position; (3) hole removed from the tip of the leaf five nodes down the stem from the newest unfolded leaf (i.e., 85–95% full expansion); (4) hole removed from the base of that leaf; (5) control plants that received no damage on any leaves and the second and fifth leaves were used as controls for the expanding and mature leaves, respectively. On damaged plants, the holes were positioned as distal (tip) or proximal (base) as possible on the leaf while still being completely within the lamina and never entering the mid-vein.

Damage-treated and control leaves were harvested 35 days after plants had been damaged, long after leaf expansion had ceased but prior to leaf senescence. All plants had produced at least 25 leaves by this time. The area occupied by the hole was determined with a leaf area meter (Li-Cor Inc. Lincoln, Neb.) by measuring the area of the leaf with the hole and subtracting that value from the area of the leaf with the hole covered with masking tape. The final area of the leaf was also recorded. After plants had completed flowering, approximately 40 days after leaves were harvested, fruits were harvested from each plant.

Mean final size of the hole, the final leaf area of the damaged and control leaves, and the number and mass of fruits produced per plant were analyzed with analysis of variance and means were compared with a Tukey's multiple range test.

Results and discussion

Predicting herbivore impacts via leaf development

The results were consistent with the hypotheses stated in the introduction. First, it was predicted that the size of the initial hole in expanding tissue should get bigger as

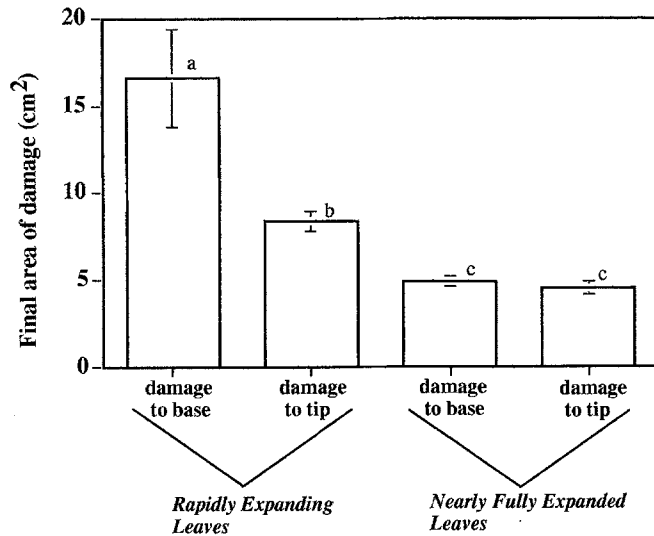


Fig. 1 The mean final area (± 1 SE; $n = 5$) of circular holes that had an initial area of 3.9 cm^2 when they were cut from the base or tip of either rapidly expanding leaves (two nodes from the apex; 20–30% of full expansion) or nearly fully expanded leaves (five nodes from the apex; 85–95% full expansion). The letters represent significance levels from a Tukey's multiple range test of means. Means by bars headed with different letters are significantly different ($P < 0.05$).

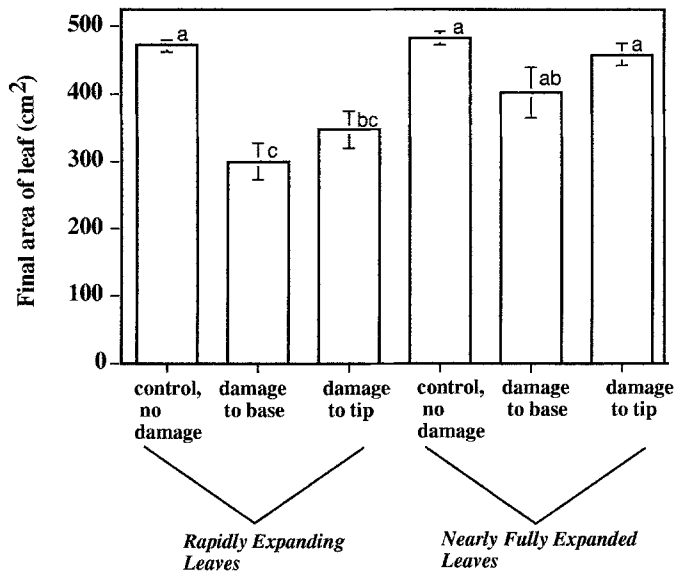


Fig. 2 Mean area (± 1 SE; $n = 5$) at maturity for leaves that were damaged at either the base or tip of the lamina during their phase of rapid expansion (20–30% full expansion; two nodes from the apex) as they neared full expansion (85–95% full expansion; five nodes from the apex) or undamaged leaves. The letters represent significance levels from a Tukey's multiple range test of means. Means represented by bars headed with different letters are significantly different ($P < 0.05$).

the cells bordering the hole expand. The average final size that an initial hole of 3.9 cm^2 reached for expanding leaves damaged at the base was 16.6 cm^2 – over 4 times the size of the initial damage and significantly greater than the final hole size on any other treatment (Fig. 1;

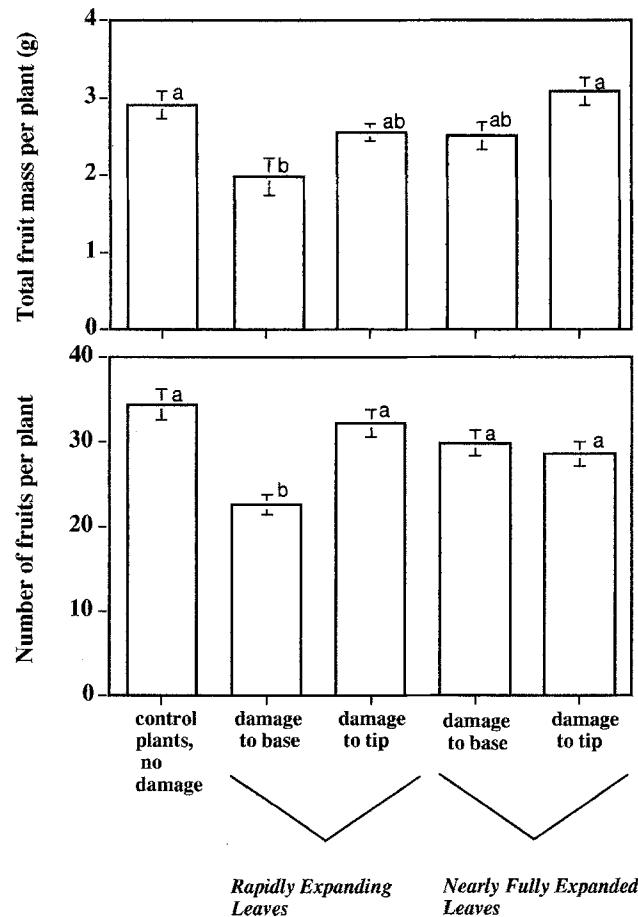


Fig. 3 Mean total fruit mass and number of fruits per plant (± 1 SE; $n = 5$) as a function of whether or not plants had a single leaf damaged by the removal of a 3.9 cm^2 hole and whether leaves were damaged at the base or tip of the lamina during their phase of rapid expansion (20–30% full expansion; two nodes from the apex) or as they neared full expansion (85–95% full expansion; five nodes from the apex). Control plants received no damage. The letters represent significance levels from a Tukey's multiple range test of means. Means represented by bars headed with different letters are significantly different ($P < 0.05$).

$P < 0.05$ Tukey's). The hole size in tips of expanding leaves nearly doubled and was significantly larger than either of the holes in mature leaves ($P < 0.05$ Tukey's), whose holes increased in area by less than 15% (Fig. 1).

We also predicted that the final area reached by leaves damaged at the base should be drastically reduced, beyond the reduction due to the size of the hole, as the removal of tissue results in the removal of both cells which were not fully expanded and intercalary meristems as well as disrupting the transport of water and carbohydrates into the leaf. This is exactly what we observed (Fig. 2). For example, expanding leaves that had a 3.9 cm^2 hole removed from the base ended up with a final mean leaf area of approximately 299 cm^2 , whereas undamaged leaves reached a final size of approximately 480 cm^2 (Fig. 2). Consequently, the removal of 3.9 cm^2 resulted in a final hole size of 16.6 cm^2 , but a reduction in leaf area relative to control plants of over 180 cm^2 –

nearly a 40% reduction in final size (Fig. 2). Similar results occurred with tip-damaged expanding leaves whose mean final size was over 25% smaller than undamaged leaves (Fig. 2). Although the mean final area of base-damaged expanded leaves was over 40 cm² less than tip-damaged expanding leaves, the difference was only significant at $0.05 < P < 0.1$ (Tukey's; Fig. 2). Analysis of variance, however, showed that damage to the base of the leaf, irrespective of its relative state of expansion, had a greater impact on final leaf area than did damage to leaf tips ($P < 0.001$). This makes sense because even in nearly expanded leaves, it is the base of the leaf which has not yet completed expansion. Damage to this tissue would therefore disrupt the final phases of leaf expansion in these nearly mature leaves as well as in rapidly expanding leaves.

Is it possible that such a small amount of damage (i.e., 3.9 cm²) to an expanding leaf could actually affect plant performance? We tested this idea by measuring the production and mass of fruits by damaged and undamaged plants. Plants with damage to the base of an expanding leaf produced a mean of 22.4 fruits per plant and a mean total fruit mass of 1.98 g – an approximate 35% reduction in both fruit number and mass in comparison to all other treatments ($P < 0.05$ Tukey's; Fig. 3). Although damage to the tip of an expanding leaf resulted in a large reduction in final leaf size, it did not result in a reduction in fruit number or mass. We do not have an explanation for this result. We also did not measure whether there were changes in the size of non-damaged leaves on damaged plants, and it is possible that the area of non-damaged leaves may have increased in a fashion that compensated for the reduction in area of the damaged leaves. However, the strong effect that damage to the base of an expanding leaf had on fruit production suggests that this was not the case.

Our conclusions are unlikely to be an artifact of the species that we used. Leaf development proceeds in an orderly and predictable fashion in all plants (Isebrands and Larson 1973; Esau 1977), and tobacco leaf development is thought to be characteristic of many dicots (Esau 1977). However, the actual patterns of leaf development do vary among plant species (Dickson and Isebrands 1991), and the position of a leaf on a stem when a certain developmental event occurs can change as a function of the effects of genotypic or environmental variation on rates of leaf production (reviewed in Lamoreaux et al. 1978). However, as patterns of leaf development always follow an orderly and predictable sequence of events, a knowledge of leaf development can be a robust predictor of how damage to a specific part of a specific leaf will affect the plant.

Implications to studies of plant-herbivore interactions

The results from this simple experiment have a number of implications for the design and interpretations of experiments investigating the nature of plant-herbivore in-

teractions. First, there has been substantial interest in determining the rates of consumption by herbivores in natural and agricultural ecosystems, as well as the amount of damage that plants sustain due to herbivory (e.g., Coley 1983; McNaughton et al. 1991). Consumption by herbivores and amounts of plant damage are generally estimated from the leaf area missing from a leaf or a plant at a single point in time (e.g., Bray 1961; Fox and Morrow 1983; see Lowman 1984) or continually on individual leaves through the season (Coley 1983; Lowman 1984; Hargrove and Crossley 1988). The data presented here suggest that either of these methods can lead to *overestimates* of consumption and *underestimates* of the effect of herbivory on leaf area display. For example, if we were to estimate the amount of leaf area consumed by herbivores from the size or growth of the hole taken from the base of an expanding leaf, we would conclude that herbivores removed 16.6 cm² even though only 3.9 cm² was actually removed. On the other hand, if we were to estimate the reduction in overall leaf area display simply from the size of the hole in a leaf, we would conclude that leaf area was reduced by 16.6 cm² when, in fact, damage to the base of the leaf resulted in over a 180 cm² reduction in the area of that leaf. We would have therefore underestimated damage to those leaves by approximately tenfold.

Second, it has been proposed that the dispersion of a given amount of herbivore damage on many leaves may result in a smaller impact on plant performance than that same amount of damage concentrated on a few leaves, and that induced defenses might result in the dispersion of damage (Edwards and Wratten 1983). Yet studies have produced inconsistent results regarding the effect of concentrated versus dispersed damage on plant performance (Lowman 1982; Wit 1982; Marquis 1992; Mauricio et al. 1993). These studies generally have not fully incorporated the notion that the location and age of a leaf and where on the leaf damage occurs, may play a huge role in the overall effect that damage has on plant performance. For example, concentrated damage at the base of an expanding leaf is shown in our study to have a very different effect than concentrated damage at the tip of that same leaf. These data also shed some light on an alternative explanation for the occurrence of dispersed damage within leaves. Herbivores can track developmental stages of leaves (e.g., Raupp and Denno 1983; Coleman and Jones 1988; Jones and Coleman 1988). Thus, damage can be dispersed within a leaf simply because herbivores track changes in tissue quality associated with the process of leaf development and expansion and not necessarily because of the induced activity of plant defenses.

Third, there has been a large number of studies examining the preference or performance of herbivores for leaves of a given age (reviewed in Raupp and Denno 1983). For example, specialist herbivores have been hypothesized to feed on young leaves because they have evolved to detoxify the species-specific chemical defenses of those leaves and can therefore utilize the higher quality tissue that these leaves represent (Cates 1981). In

most studies of this phenomenon, leaf age is classified by relative position on the stem or by chronological age (reviewed in Coleman 1986). However, it is clear from both this study and other studies (e.g. Isebrands and Larson 1973) that expanding leaves, both within and among themselves, are tremendously heterogeneous. Differences in the anatomy, physiology, and biochemistry between the tip and base of a given expanding leaf can be much greater than between mature leaves that are separated by several nodal positions on the stem (Coleman 1986). Thus, it is clear that an understanding of leaf development and maturation needs to be incorporated into studies examining the ecological and evolutionary consequences of leaf aging to herbivores.

In conclusion, we showed that the location on a leaf where a herbivore feeds, and the developmental stage of a leaf that herbivore feeds upon, has consequences for the amount of area that is eventually lost to a herbivore feeding event, the final area that a leaf can reach after tissue has been removed by herbivores, and the reproductive output of the plant. Thus, it clearly matters where on the leaf a herbivore feeds, and understanding the basics of leaf development enables one to predict better how and when it will matter.

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References

- Aide TM, Londoño EC (1989) The effects of rapid leaf expansion on the growth and survivorship of a lepidopteran herbivore. *Oikos* 55: 66–70
- Avery GS (1933) Structure and development of the tobacco leaf. *Am J Bot* 20: 565–592
- Bray JR (1961) Measurement of leaf utilization as an index of minimum levels of primary consumption. *Oikos* 12: 70–74
- Cates RG (1981) Feeding patterns of monophagous, oligophagous and polyphagous insect herbivores: the effect of resource abundance and plant chemistry. *Oecologia* 46: 22–31
- Coleman JS (1986) Leaf development and leaf stress: increased susceptibility associated with sink-source transition. *Tree Physiol* 2: 289–299
- Coleman JS, Jones CG (1988) Acute ozone stress on eastern cottonwood (*Populus deltoides* Bartr.) and the pest potential of the aphid, *Chaitophorus populicola* Thomas (Homoptera: Aphididae). *Environ Entomol* 17: 207–212
- Coleman JS, Jones CG (1991) A phytocentric perspective of phytochemical induction by herbivores. In: Tallamy D, Raupp M (eds) *Phytochemical induction by herbivores*. Wiley, New York, pp 3–43
- Coleman JS, Jones CG, Krischik VA (1992) Phytocentric and exploiter perspectives of phytopathology. *Adv Plant Pathol* 9: 149–195
- Coley PD (1983) Herbivory and defensive characteristics of tree species in lowland tropical forests. *Ecol Monogr* 53: 209–233
- Cranshaw WS, Radcliffe EB (1980) Effect of defoliation on the yield of potatoes. *J Econ Entomol* 73: 131–134
- Dickson RE, Isebrands JG (1991) Leaves as regulators of stress response. In: Mooney HA, Winner WE, Pell EJ (eds) *Response of plants to multiple stresses*. Academic Press, San Diego, pp 4–34
- Edwards PJ, Wratten SD (1983) Wound induced defenses in plants and their consequences for patterns of insect grazing. *Oecologia* 56: 88–93
- Esau, K (1977) *Anatomy of seed plants*, 2nd edn. Wiley, New York
- Fox LR, Morrow PA (1983) Estimates of damage by herbivorous insects on *Eucalyptus* trees. *Aust J Ecol* 8: 139–149
- Hargrove WW, Crossley DA (1988) Video digitizer for the rapid measurement of leaf area lost to herbivorous insects. *Ann Entomol Soc Am* 81: 593–598
- Harper JL (1989) The value of a leaf. *Oecologia* 80: 53–58
- Isebrands JG, Larson PR (1973) Anatomical changes during leaf ontogeny in *Populus deltoides*. *Am J Bot* 60: 199–208
- Jones CG, Coleman JS (1988) Plant stress and insect behavior: cottonwood ozone and the feeding and oviposition preference of a beetle. *Oecologia* 76: 51–56
- Lamoreaux RJ, Chaney WR, Brown KM (1978) The plastochron index: a review after two decades of use. *Am J Bot* 65: 586–593
- Lowman MD (1982) Effects of different rates and methods of leaf area removal on rain forest seedlings of coachwood. *Aust J Bot* 30: 477–483
- Lowman MD (1984) An assessment of techniques for measuring herbivory: is rainforest defoliation more intense than we thought? *Biotropica* 16: 264–268
- Marquis RJ (1992) A bite is a bite? Constraints on response to folivory in *Piper arieianum* (Piperaceae). *Ecology* 73: 143–152
- Mauricio R, Bowers MD, Bazzaz FA (1993) Pattern of leaf damage affects fitness of the annual plant *Raphanus sativus* (Brassicaceae). *Ecology* 74: 2066–2071
- McKey D (1979) The distribution of secondary metabolites within plants. In: Rosenthal GA, Janzen DH (eds) *Herbivores: their interaction with secondary metabolites*. Academic Press, New York, pp 55–133
- McNaughton SJ, Oesterheld M, Frank DA, Williams KJ (1991) Primary and secondary production in terrestrial ecosystems. In: Cole J, Lovett G, Findlay S (eds) *Comparative analyses of ecosystems: patterns, mechanisms and theories*. Springer, New York, pp 120–139
- Mooney HA, Gulmon SL (1982) Constraints on leaf structure and function in reference to herbivory. *BioScience* 32: 198–206
- Raupp M J, Denno RF (1983) Leaf age as a predictor of herbivore distribution and abundance. In: Denno RF, McClure MS (eds) *Variable plants and herbivores in natural and managed systems*. Academic Press, New York, pp 91–125
- Stamp NE, Bowers MD (1990) Phenology of nutritional differences between new and mature leaves and its effect on caterpillar growth. *Ecol Entomol* 15: 447–454
- Wit AKH (1982) The relationship between artificial defoliation and yield in Brussels sprouts as a method to assess the quantitative damage induced by leaf eating insects. *Z Angew Entomol* 94: 425–431