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Pattern of defoliation and its effect on photosynthetic capacity in *Oenothera biennis*

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Summary

1 Five different patterns of tissue removal were used to mimic natural herbivore damage in *Oenothera biennis*. The effects of defoliation by: (1) half edge (tissue removed from one side of the leaf, parallel to and exclusive of the midrib); (2) double edge (tissue removed from leaf perimeter); (3) perforation (roughly circular portions removed, leaf edges and midrib intact); (4) tip (apex of leaf removed); and (5) entire leaf defoliation on photosynthesis were examined.

2 Plants were subjected to removal of tissue from either a single leaf or throughout the plant. Photosynthesis, stomatal conductance, nitrogen content and water potential of the remaining portion of the youngest fully emerged leaf at the time of defoliation was examined over a one to two week period.

3 Tip defoliation at both the single leaf and whole plant level enhanced photosynthesis relative to undefoliated controls. Half edge and entire leaf defoliation at the whole plant level also enhanced photosynthesis relative to controls, but not to the same extent as tip defoliation. Double edge and perforation defoliation at the single leaf level depressed photosynthesis relative to controls and tip defoliation.

4 Differences among defoliation treatments can best be explained by intraleaf variation in photosynthetic capacity and nitrogen content and by the extent of wounding to residual tissue. Results from this study illustrate that the way in which tissue is removed can have a dramatic effect upon photosynthetic capacity of the remaining tissue.

Keywords: herbivory, level of defoliation, nitrogen content, *Oenothera biennis*, pattern of defoliation, photosynthesis, stomatal conductance, water potential

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Introduction

A controversy has arisen concerning the effect of herbivory upon plant fitness (Belsky 1986). Many studies show that herbivory has detrimental effects upon plant growth and reproduction (e.g. Mattson & Addy 1975; Morrow & LaMarche 1978; Marshall 1989; Mihaliak & Lincoln 1989; Welter 1991), while other studies demonstrate beneficial effects (e.g. Paige & Whitham 1987; Strauss 1988) and still others demonstrate variable effects (e.g. Maschinski & Whitham 1989; Gedge 1992).

Herbivory can be beneficial both at the level of the population and the individual. At the population level for example, herbivory can remove competitors less capable of tolerating or resisting herbivory (Crawley 1989). At the individual level, herbivory can indirectly influence factors external to the plant such as light, water and nutrient availability and so affect photosynthesis and consequently, growth. It can also alter photosynthesis directly through changes in source–sink relations and rate of tissue senescence, or through the effects of hormones secreted by herbivores (McNaughton 1984; Senock *et al.* 1991; Hoogesteger & Karlsson 1992).

Given that the effect of herbivory upon photosynthetic capacity appears to be highly variable, the objective of our study was to examine some possible factors which could contribute to this variation. Depending upon factors such as herbivore body size, mouth-part structure, and within-plant variation in food quality, herbivory can result in very different patterns of defoliation. Herbivores may remove tissue in disc-like pieces scattered throughout a leaf, or consume either the tip or outer portions of a leaf, or entire leaves. Could postdefoliation photosynthesis be influenced by pattern of tissue removal?

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Marshall (1989) reported that for two *Sesbania* species, removal of every other leaf vs. removal of half of each leaf can have different consequences. Removal of every other leaf from *S. macrocarpa* has a more damaging effect upon reproduction than does the removal of half of each leaf, but in *S. vesicaria*, total seed mass and total mass of seeds per fruit were higher in plants with every other leaf removed than with half of each leaf removed. Marquis (1992) demonstrated that the distribution of damage within *Piper arieianum* affected subsequent growth and reproduction. Removal of 10% of the total leaf area scattered throughout the canopy did not significantly reduce seed production, whereas localized damage (10% of total leaf area removed from a single branch) led to diminished growth and seed production at the branch and whole plant level.

The present study examines the relationship between photosynthesis and pattern of leaf tissue removal in the short-lived monocarpic perennial, *Oenothera biennis* (L.). This species forms a vegetative rosette in its first year and produces a reproductive spike, usually in its second year of growth (Hall *et al.* 1988). Preliminary field studies with this species indicate that both vegetative and reproductive plants are subjected to substantial levels of leaf defoliation (Morrison, unpublished data). At least five more or less distinct patterns of natural herbivore damage can be observed: (1) half edge, whereby half of the leaf tissue is removed parallel to, but exclusive of the midrib; (2) double edge, partial tissue damage to entire leaf perimeter; (3) perforation, roughly circular portions removed, leaf edges and midrib intact; (4) tip, apex of leaf consumed, often approaching leaf midpoint (Fig. 1); and (5) entire leaf defoliation.

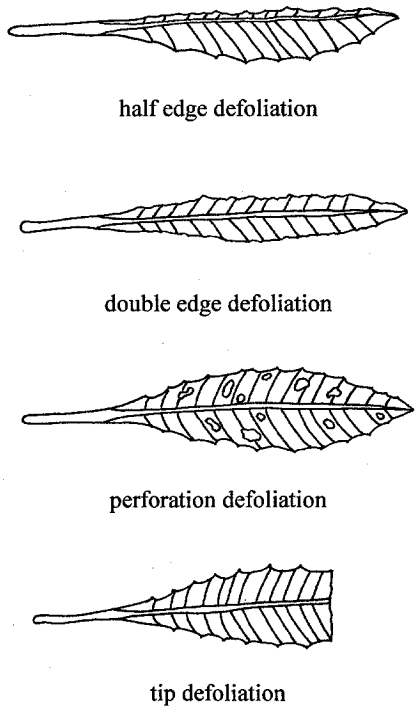

Fig. 1 Representative patterns of tissue removal in field collected *Oenothera biennis* leaves.

This study simulates each pattern of leaf damage in a series of experiments to compare the photosynthetic rate, stomatal conductance, nitrogen content and water potential of residual tissue to that of undefoliated controls. The first experiment examines the effect of tip, half edge and entire leaf defoliation when 50% of total plant leaf area is removed. A second experiment examines the effect of double edge and perforation defoliation when 30% of a single leaf is removed. As defoliation had very different effects upon photosynthetic rate in these two experiments, expt 3 examines how differences in level of defoliation affects response. Experiment 4 compares tip and perforation defoliation in a single experiment at the same level of defoliation. In addition, expt 4 examines how variation in the degree of wounding associated with different types of defoliation affects photosynthesis of residual tissue. Finally, expt 5 examines intraleaf variation in photosynthesis in intact leaves to determine if such variation could account for some of the differences in the effects of different defoliation patterns.

Materials and methods

PLANT CULTURE

Naturally stratified seeds were collected in spring 1992 from spikes produced the previous year. For expts 1 and 2, seeds were germinated in glass Petri dishes containing distilled water-saturated filter paper. Upon cotyledon emergence, seedlings were transplanted into 10-cm pots, containing 0.35 L of a 1:1 mixture of sand and a peat-based commercial potting soil. After the first 5 weeks of growth, plants were fertilized once every 2 weeks with Plant-Prod 10–52–10 fertilizer at a rate of 5 mL L^{-1} H_2O (Plant Products Co. Ltd, Brampton, ON). For expts 3, 4 and 5, seeds were germinated in plastic flats containing a peat based soil mix. Seven days after emergence, seedlings were transplanted into a 72-plug tray containing the same soil mixture (4 cm^3 per seedling). Following a further 33 days of growth, plants were repotted into either 10- or 15-cm pots containing 0.35 or 1.2 L, respectively, of a 1:1 mixture of sand and a peat based commercial potting soil. Depending upon pot size, soil was mixed with either 2 or 6 g of a 3–4 month formulation of Osmocote 14–14–14 controlled release fertilizer (Osmocote, Milpitas, CA). Plants in all five experiments were grown in a glasshouse receiving natural light levels and photoperiod (May–August). Plants were rotated among positions once a week.

EXPERIMENT 1

Eighty five days after germination, the youngest fully emerged leaf (YFEL) was selected in each of 48 plants,

and marked at the base of the petiole with a nontoxic permanent marker for future identification. Twelve plants were randomly assigned to each of the following four treatments: tip, half edge and entire leaf defoliation and undefoliated controls.

Each defoliation treatment involved removing approximately half of the total plant leaf area. Tip defoliation removed half the length of every leaf on the plant (including the YFEL) starting from the tip. Half edge defoliation removed half of every leaf on one side parallel to the midrib which was left intact. Both patterns of tissue removal are depicted in Fig. 1. Entire leaf defoliation removed alternate leaves with the YFEL left intact.

Prior to defoliation, the length and width of each leaf on every plant was recorded. A linear relationship between leaf area and the product of leaf length × width was derived using detached leaves from the entire leaf defoliation treatment. These data, along with the area of leaf tissue removed, were used to calculate the actual proportion of total plant leaf area removed in each defoliation treatment. Leaf area measurements were made with the LI-COR model LI-3100 leaf area meter (LI-COR Inc., Lincoln, NB) in the high resolution mode.

Gas exchange measurements were made on days 1, 6 and 14 postdefoliation. In the early morning, plants were placed on tables located in an open field near the glasshouse for acclimatization. Measurements were only made on clear, sunny days. Photosynthetic photon flux ranged between 1100 and 1600 Nmol m^{-2} s^{-1}, temperature ranged between 27 and 33 °C, and vapour pressure varied between 1.7–2.6 kPa.

Gas exchange measurements began mid-morning and were completed within a 4-h period. Net photosynthesis and stomatal conductance of the previously marked leaves were determined using the LI-COR LI-6250 portable gas exchange system with the LI-COR model 6000-10 4-L leaf chamber (LI-COR Inc., Lincoln, NB). The remaining portion of the YFELs was placed completely within the chamber. The system was calibrated daily using a 350-μL CO_2 L^{-1} airstream provided by a LI-COR model 6000-01 gas mixing cylinder. The flow of chamber air through the desiccant column was predetermined for each treatment using representative leaves so that changes in vapour pressure during measurements were negligible. Measurements averaged 2 min and leaf temperatures varied less than 2 °C over the course of a measurement. Immediately following gas exchange measurements, the leaf area contained within the chamber was determined by tracing leaves onto clear, plastic film and measuring with a leaf area meter.

EXPERIMENT 2

Ninety-one days after germination, 12 plants were randomly assigned to each of the following three treatments: double edge and perforation defoliation, and undefoliated controls. The YFEL on each plant was marked and traced to determine leaf area. The number of paperpunch perforations (6.0 mm in diameter) required to remove ≈ 30% of the YFEL area was calculated and tissue was removed in one of two ways: (1) for double edge defoliation, tissue was removed in hemispherical portions (half the paperpunch area) around the entire YFEL margin, and (2) for perforation defoliation, tissue was punched from within the YFEL leaving the midrib and leaf edges intact (see Fig. 1).

Gas exchange measurements of marked leaves were made on days 1, 9 and 12 postdefoliation. Procedures followed those of the preceding experiment. Due to leaf mortality, it was not possible to take gas exchange measurements for several plants on days 9 and 12 of the experiment, i.e. $n = 10$, 7 and 7 on day 9 and $n = 10$, 5 and 6 on day 12 for controls, double edge and perforation treatments, respectively.

EXPERIMENT 3

Sixty-six days after germination, plants growing in 10-cm pots were randomly assigned to one of the following four treatments: undefoliated controls and low, medium and high levels of entire leaf defoliation. Eight plants were assigned to the medium level of defoliation and 17 plants each to the remaining treatments. Leaves were removed beginning with the next to youngest leaf and according to the patterns: low, remove one leaf skip the next two; medium, remove every other leaf; and high, remove two leaves skip the next. In each case therefore, the YFEL was left intact and marked for future identification. By determining both the amount of leaf area removed and the amount remaining, the exact level of defoliation was calculated as described for expt 1.

Gas exchange measurements of marked leaves were made on days 1, 7 and 14 postdefoliation. Plants were allowed at least 1 h for acclimatization within a controlled environment growth chamber that provided a photosynthetic photon flux of 700 μmol m^{-2} s^{-1} and a temperature of 22 °C. After acclimatization, photosynthesis and stomatal conductance were measured with the LI-COR gas exchange system as described above. Initially, the chamber was placed in the open mode to allow the leaf to come into equilibrium with chamber conditions and to adjust flow through the desiccant column to minimize changes in vapour pressure, then placed in the closed mode to take the measurement. Air from outside the building was pumped into the growth chamber to prevent any increase in CO_2 levels due to breathing by the operator. Gas exchange measurements began mid-morning and were completed within a 6-h time frame. Temperatures within the leaf chamber varied less than 0.5 °C over the course of any individual measurement. Depending upon the leaf, temperatures ranged between 24 and 26 °C.

On day 1 postdefoliation, water potential and nitrogen content was determined using nine plants from each of the low and high defoliation treatments and undefoliated controls. On day 14, measurements were taken for the remaining plants from all four treatments. Water potential of marked leaves was determined immediately after gas exchange measurements using a Decagon model SC-10a thermocouple psychrometer (Decagon Devices Inc., Pullman, WA). Detached leaves were placed in psychrometer cups and allowed to equilibrate within the system for at least 30 min. These leaves were then dried in an oven at 45 °C for more than 48 h and their nitrogen content determined using a LECO model CHN-1000 carbon–hydrogen–nitrogen analyser (LECO Corp., St. Joseph, MI).

EXPERIMENT 4

Seventy-five days after germination, 11 plants (10-cm pots) were randomly assigned to each of five treatments: small (3 mm in diameter) and large perforations (6 mm), tip defoliation, sliced tissue, and undefoliated controls. The YFEL was marked on each plant and ≈ 30% of its tissue was removed in each of the defoliation treatments. However, length of the cut edge differed among treatments. Based on calculations of the summed perimeter of large vs. small perforations, the large perforation treatment had a cut edge of 1340 mm and the small perforation treatment had a cut edge of 2460 mm. Tip defoliation wounded approximately 40 mm of tissue (i.e. the width of the leaf blade). The YFEL of the sliced tissue treatment was cut parallel to the lateral veins (midrib and edges intact) so that the length of leaf tissue damaged equalled that of the large perforation treatment without actual tissue removal.

Gas exchange measurements were made on days 1, 4 and 7 postdefoliation following the procedures outlined in expt 3.

EXPERIMENT 5

Seventy-two days after germination, the YFEL of 20 plants (15-cm pots) was marked in thirds lengthways from the leaf tip to the very base of the leaf blade and termed tip, mid, and base, respectively. Photosynthesis and stomatal conductance of each of these sections was then determined as described for expt 3 except that here, the LI-COR model 6000-11 0.25-L leaf chamber was used to prevent leaf section overlap. Upon completion of gas exchange measurements, the YFELs were removed and dried at 45 °C for nitrogen content analysis.

To examine intraleaf variation in water potential, an additional 10 plants were chosen from those grown in 15-cm pots. The YFEL on each plant was marked as described above. Plants were well watered the day before measurements were made, but were not watered on the day of measurements. On the morning of the experiment, plants were placed in a growth chamber which provided an irradiance of 700 μmol m^{-2} s^{-1} and a temperature of 22 °C. Water potential measurements began mid-afternoon, i.e. 7 h after chamber lights came on. The innermost portion (12 mm × 45 mm) of the tip, mid, and base sections for each YFEL were removed and allowed to equilibrate within the closed chamber of the thermocouple psychrometer.

STATISTICAL ANALYSES

For expts 1–4, data were analysed by means of a one-way analysis of variance with herbivory type as a fixed effect in a completely random experimental design. Data for the different measurement days were analysed separately. Data from expt 5 were analysed by means of analysis of variance with leaf section as a fixed effect in a randomized complete block design and individual leaves as blocks. In cases where there was a significant F-test for the treatment effect, means were compared using the Student–Newman–Keuls test. Temporal trends within experiments were analysed by means of a repeated measures analysis of variance with herbivory type and time as fixed effects. Hypotheses regarding temporal trends were tested using the Wilks' Lambda F Statistic. Unless otherwise stated, the 0.05 level of probability was used for all tests of significance.

Results

EXPERIMENT 1

This experiment was designed to remove approximately half the leaf area from individual plants using three different defoliation patterns. The actual level of defoliation achieved was 46% for tip, 41% for half edge and 51% for entire leaf defoliation.

Defoliation enhanced photosynthetic rate regardless of the pattern of leaf removal (Fig. 2A, MANOVA treatment effect: $P = 0.001$, $F_{3,41} = 25.3$). On day 1, only tip defoliation was higher than controls. However, by day 6, all defoliated treatments had higher photosynthetic rates than controls and tip defoliated plants were significantly higher than those of other defoliation treatments. The differences between defoliated and control plants increased further by day 14. Control photosynthetic rates declined and then became constant over time while rates of defoliated plants increased with time (MANOVA time treatment interaction: $P = 0.001$, $F_{6,82} = 7.87$).

Initially, stomatal conductance varied little among treatments but, as the experiment progressed, conductance of the defoliated treatments increased while that of the control remained more or less constant (Fig. 2B, MANOVA time–treatment interaction: $P = 0.0087$, $F_{6,82} = 3.11$). On day 1 only half edge

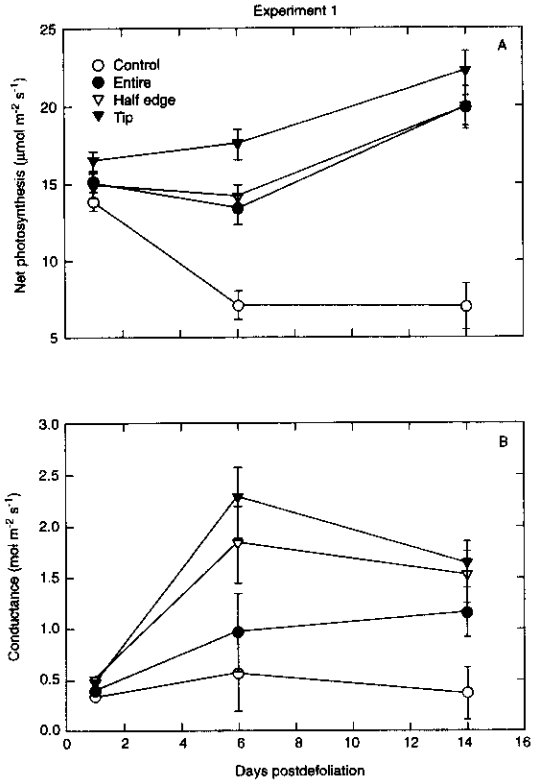

Fig. 2 Mean photosynthetic rate (A) and stomatal conductance (B) of youngest fully emerged leaves for undefoliated controls, tip, half edge and entire leaf defoliation in expt 1. Total plant leaf area removed was 46, 41, and 51% for tip, half edge and entire leaf defoliation, respectively. Error bars represent one standard error.

defoliation was significantly higher than that of controls. Tip defoliation had the highest conductance values, and was significantly different from those treatments with the YFEL intact (i.e. entire leaf defoliation and control) on days 6 and 14. Ultimately, all defoliated treatments had conductance values greater than those of the controls.

EXPERIMENT 2

There was relatively little variation in photosynthetic rate among treatments in this experiment (Fig. 3A). Perforation and double edge tissue removal in the YFEL led to an initial depression in photosynthetic rate (univariate ANOVA treatment effect: $P = 0.0048$, $F_{2,33} = 6.30$). However, this difference disappeared entirely by day 9. There was no significant difference in photosynthetic rates over time for any of the treatments.

Stomatal conductance did not vary significantly among treatments or with time (Fig. 3B).

EXPERIMENT 3

The actual level of tissue removed in the low, medium and high entire leaf defoliation treatments averaged 29, 43 and 60%. All treatments experienced a continuous decline in net photosynthesis over the course of the experiment; however, treatments declined at different rates (Fig. 4A, MANOVA time–treatment interaction: $P = 0.0045$, $F_{6,54} = 3.61$). The defoliated treatments declined to a lesser extent than the controls. There were never any significant differences among levels of defoliation, only between defoliation treatments and the control.

Conductance peaked on day 7 for all treatments then declined (Fig. 4B, MANOVA time effect: $P = 0.0001$, $F_{2,54} = 205$). There were no differences among treatments in conductance except on day 14 where medium and high levels of defoliation had higher conductance relative to undefoliated controls (univariate ANOVA treatment effect: $P = 0.0142$, $F_{3,27} = 4.23$).

Water potential of the YFEL (Table 1) did not vary with level of defoliation on either day 1 or 14. Immediately following defoliation, i.e. day 1, nitrogen levels were markedly higher in the defoliated treatments than in the control (Table 1, univariate ANOVA treatment effect: $P = 0.0017$, $F_{2,23} = 8.53$); however, there were no differences between levels of defoliation. By day 14 of the experiment, nitrogen content within all treatments had dropped below previous values (MANOVA time effect: $P = 0.001$, $F_{1,20} = 100$), but the YFEL of the medium and high defoliation treatments still contained significantly higher amounts of nitro-

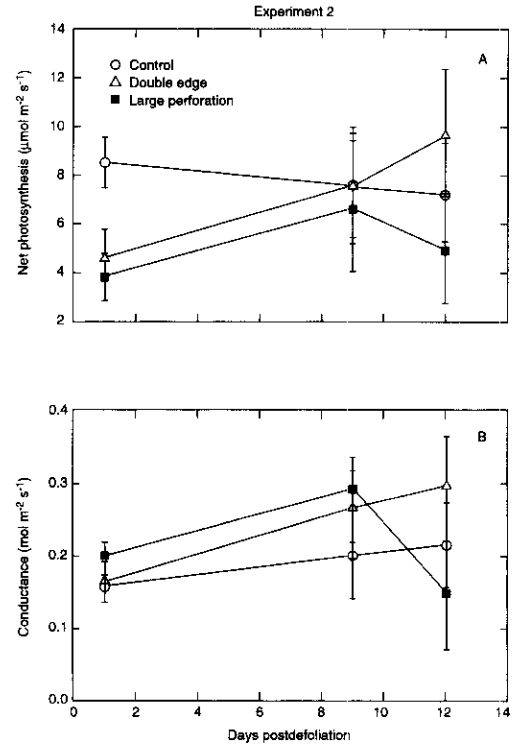

Fig. 3 Mean photosynthetic rate (A) and stomatal conductance (B) of youngest fully emerged leaves (YFEL) for undefoliated controls, perforation and double edge defoliation in expt 2. Approximately 30% of the area of the YFEL was removed in each defoliation treatment. Error bars represent one standard error.

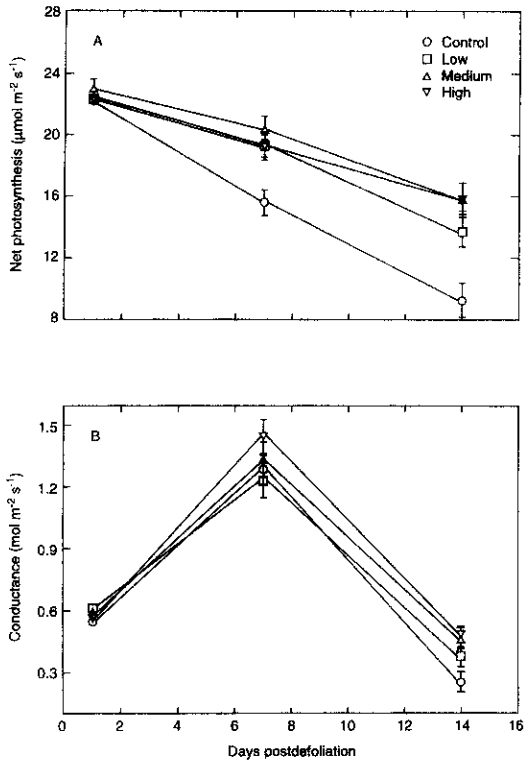

Fig. 4 Mean photosynthetic rate (A) and stomatal conductance (B) of youngest fully emerged leaves for undefoliated controls, low (29%), medium (43%) and high (60%) levels of whole plant defoliation in expt 3. Defoliation was by means of entire leaf removal. Error bars represent one standard error.

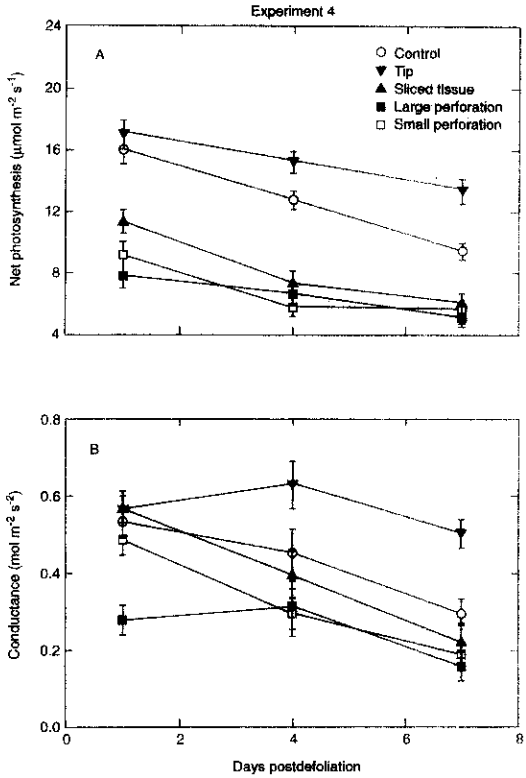

Fig. 5 Mean photosynthetic rate (A) and stomatal conductance (B) of youngest fully emerged leaves (YFEL) of undefoliated controls, sliced tissue, tip, small large perforation defoliation in expt 4. Approximately 30% of the area of the YFEL was removed in each defoliation treatment. Error bars represent one standard error.

gen than the controls (univariate ANOVA treatment effect: $P = 0.0013$, $F_{3,28} = 6.91$).

EXPERIMENT 4

Net photosynthesis declined over time in all treatments (Fig. 5A, MANOVA time effect: $P = 0.0001$, $F_{2,98} = 86.0$) but the rate of decline differed among treatments (MANOVA time–treatment effect: $P = 0.0041$, $F_{8,98} = 3.06$). Tip defoliation was the only treatment to exhibit enhanced photosynthetic rates relative to the control with the magnitude of this effect increasing over time. The remaining treatments had rates which were always depressed compared to those of the control and tip defoliation. On day 1, the sliced tissue treatment had a higher photosynthetic rate than the large perforation treatment.

In general, conductance declined over time (MANOVA time effect: $P = 0.0001$, $F_{2,98} = 66.2$) but there was some variation in this pattern among treatments (MANOVA time–treatment interaction: $P = 0.0002$, $F_{8,98} = 4.31$). Leaves with large perforations had the lowest stomatal conductance of all treatments on the day immediately following wounding (Fig. 5B). Tip defoliation had the highest conductance values for days 4 and seven postdefoliation. There were no other differences among treatments in stomatal conductance.

Table 1 Mean water potential and nitrogen content (± SE) of youngest fully emerged leaves on days 1 and 14 postdefoliation in expt 3. Defoliation was by removal of entire leaves. The proportion of total leaf area removed was 0, 29, 43 and 60% for control, low, medium and high levels of defoliation. Standard errors are respective to column values

	Defoliation level			
	Control	Low	Medium	High
Water potential (kPa)				
Day 1	−0.13 ± 0.01	−0.12	—	−0.12
Day 14	−0.12 ± 0.01	−0.12	−0.12	−0.13
Nitrogen content (%)				
Day 1	2.59 ± 0.25	3.42	—	3.99
Day 14	1.35 ± 0.09	1.56	1.86	1.84

EXPERIMENT 5

There was significant intraleaf variation in photosynthetic rate (univariate ANOVA treatment effect: $P = 0.0073$, $F_{2,38} = 21.3$), stomatal conductance (univariate ANOVA treatment effect: $P = 0.0001$,

$F_{2,38} = 11.4$) and nitrogen content (univariate ANOVA treatment effect: $P = 0.0001$, $F_{2,30} = 23.1$). Values for all three parameters were highest in the midsection of YFEL (Table 2). Values for these three parameters did not differ between the base and tip portions of the YFEL. Water potential measurements revealed that the tip, mid and base sections of the YFEL were not significantly different from each other (Table 2).

Discussion

The most striking result of this study was that certain defoliation treatments (i.e. tip, half edge and entire leaf) enhanced photosynthesis of residual tissue while others (i.e. double edge, large and small perforation) depressed photosynthesis relative to controls. These differences can be seen most clearly in expt 4 (Fig. 5A). They are also evident in comparing the results of expts 1 (Fig. 2A) and 3 (Fig. 4A) to those of expt 2 (Fig. 3A) in spite of the differences in level of defoliation among experiments.

It is important to note that the age of plants (days postgermination) varied among experiments. In concordance with this, control photosynthetic rates also varied among experiments in that younger plants had higher photosynthetic rates than those plants used at a later date. Since expt 2 was conducted using the oldest plants, differences among treatments would perhaps have been more distinct if this experiment had been performed earlier in plant development when photosynthetic rates were higher.

Dramatic differences in the effect of defoliation on photosynthesis appear to be related in part, to differences in the amount of wounding associated with defoliation. The three types of defoliation that increased photosynthesis caused minimal damage to the remaining tissue. The length of the cut edge was equal to the width of the leaf blade (*c.* 40 mm), the length of the leaf (*c.* 130 mm), or the width of the petiole (*c.* 5 mm) for tip, half edge and entire leaf defoliation, respectively. On the other hand, the length of the cut edge for double edge and large and small perforation defoliation was ≈ 1340, 1340 and 2460 mm, respectively.

Wounding without tissue removal in expt 4 had essentially the same effect as the large perforation treatment (Fig. 5A), thereby supporting the suggestion that extent of wounding is important in determining response of photosynthesis to defoliation. However, there was no significant difference in response to large vs. small perforation defoliation (Fig. 5A) in spite of the difference in length of cut edge between these two treatments. It is possible that the level of damage induced by large perforation defoliation was so great, that further damage had no effect; thus, the wounding response may have been saturated.

Increasing the length of cut edge will depress photosynthesis in residual tissue for several reasons: (1) wounded tissue will have a reduced photosynthetic rate, (2) wound repair processes will require the mobilization of resources, and so deprive adjacent tissues of such resources, and (3) wounding severs vascular connections and therefore reduces water availability in adjacent tissues and the extent to which these tissues can export the products of photosynthesis.

It is not possible on the basis of the present experiments to come to a definitive conclusion regarding the relative importance of these factors. Nonetheless, the fact that photosynthetic depression disappeared by the end of the experiment (Fig. 3A), suggests that severing of vascular connections was not the most significant factor. In fully emerged leaves, it is unlikely that new vascular connections could be formed to repair the damage. It should also be noted that there was no evidence that defoliation induced stomatal closure in these experiments (Figs 3B and 5B) which would be expected if water availability was reduced due to severing of vascular connections.

The substantial photosynthetic enhancement that was observed in the half edge, entire leaf and tip defoliation treatments in expts 1, 3 and 4 (Figs 2A, 4A and 5A) was probably due in large part to the increase in root:shoot ratio associated with defoliation. Increasing root:shoot ratio enhances photosynthesis by affecting source–sink relationships, growth regulator concentrations and the availability of water and mineral nutrients (e.g. McNaughton 1983; Kim *et al.* 1991; Evans 1991; Senock *et al.* 1991).

In the present study, there was little evidence that photosynthetic enhancement was due to increased

Table 2 Mean photosynthesis, stomatal conductance, water potential and nitrogen content (± SE) for tip, mid, and base sections of youngest fully emerged leaves in expt 5. Standard errors are respective to row values

	Leaf section		
	Tip	Mid	Base
Photosynthesis (nmol m^{-2} s^{-1})	17.2 ± 0.4	19.2	18.1
Stomatal conductance (mol m^{-2} s^{-1})	0.734 ± 0.024	0.891	0.778
Water potential (kPa)	−0.13 ± 0.01	−0.12	−0.13
Nitrogen content (%)	5.08 ± 0.04	5.55	5.18

water availability. Although some of the defoliation treatments did increase stomatal conductance (Figs 2B, 4B and 5B), the increase in conductance was not due to an increase in leaf water potential (Table 1). These findings concur with a study by Wallace *et al.* (1984) whereby stimulated photosynthesis of three graminoid species was related to increased stomatal opening. Senock *et al.* (1991) demonstrated that compensatory photosynthesis of *Sporobolus flexuosus* (Thurb.) Rydb. was not due to changes in conductance or improvements in plant water status.

There was evidence that changes in nutrient availability were involved in photosynthetic enhancement. In expt 3, leaves from defoliated plants had higher nitrogen contents than undefoliated controls, both immediately following defoliation and at the end of the experiment (Table 1). A rapid increase in percentage nitrogen after defoliation may represent a decrease in carbohydrate levels due to altered source–sink relationships, rather than an increase in the absolute nitrogen content (Hoogesteger & Karlsson 1992). Nevertheless, these results are suggestive, particularly when one considers that the degree of photosynthetic enhancement increased over time (Figs 2A, 4A and 5A).

If photosynthetic enhancement was due simply to changes in source–sink relationships, then degree of enhancement should be greatest immediately following defoliation, but prior to regrowth. On the other hand, if photosynthetic enhancement was due to increased nitrogen availability, a time lag would be expected. In the present study, photosynthesis declined over time in undefoliated controls while photosynthesis was maintained in defoliated treatments. In other words, defoliation retarded the normal rate of leaf senescence. The reduction in rate of leaf senescence can be explained by an increase in nutrient availability which would reduce the need to recycle nutrients from older leaves to supply new growth.

Even among those treatments in which photosynthetic enhancement was observed, there was significant variation in extent of this enhancement; tip defoliation exhibited greater enhancement than either half edge or entire leaf defoliation (Fig. 2A). This effect cannot be attributed to the slight differences in level of defoliation achieved for these treatments. Tip defoliation (46%) was intermediate between half-edge (41%) and entire leaf defoliation (51%) in amount of tissue removed. Furthermore, expt 3 demonstrates that larger differences in level of defoliation (29–60%) had no significant effect on degree of enhancement (Fig. 4A).

Experiment 5 demonstrated that photosynthetic capacity and stomatal conductance was highest in the mid-section and lowest in the tip (Table 2). The lower photosynthetic capacity of the tip could not be explained by differences in water potential, but may be related to differences in nutrient content or rate of senescence in different parts of the leaf. Studies of leaf ontogeny indicate that it is often the leaf tip which matures first (Isebrands & Larson 1973). Therefore, it can be expected that it would senesce earlier than other parts of the leaf. In the present study, the tip section showed the first visual signs of senescence. The fact that intraleaf variation exists may explain the differences among half edge, entire leaf and tip defoliation in degree of photosynthetic enhancement. Tip defoliation in expt 5 (30% of YFEL removed) removed only tissue from the tip section, and in expt 1 (46% total leaf area removed), the majority of the tissue removed was from the tip section. Therefore, regardless of any other effects, removal of the tip will increase photosynthetic rate of the leaf.

Both photosynthetic rate and area of remaining leaf tissue were known; thus, in experiments involving single leaf defoliation (expts 2 and 4), an estimate of the impact of pattern of defoliation upon whole-leaf photosynthesis can be made. In both expts 2 and 4, on any measurement day, photosynthesis of control leaves exceeded that of the defoliated treatments. For expt 2, photosynthesis averaged 115, 77 and 45 μmol s^{-1} $leaf^{-1}$ for control, double edge and perforation treatments. Photosynthesis of leaves in expt 4 averaged 373, 260, 249, 113 and 61 μmol s^{-1} $leaf^{-1}$ for controls, tip, sliced tissue and, large and small perforation treatments, respectively. Although photosynthetic enhancement in terms of photosynthesis per unit area was observed in the tip defoliation treatment of expt 4, full compensation by the residual tissue did not occur. Other studies have also found that an increase in net photosynthesis of defoliated plants cannot compensate for the assimilate lost by removal of tissue (Chapman *et al.* 1990; Evans 1991; Hoogesteger & Karlsson 1992). It has been suggested that full compensation is more likely to occur when defoliation modifies the environment by reducing competition, increasing nutrient availability, and optimizing leaf area index, or when there are herbivore-induced hormonal alterations (McNaughton 1983, 1984; Belsky 1986; Maschinski & Whitham 1989). In the present study, plants were grown as isolated individuals in pots and defoliation is unlikely to have had a substantial effect on the external environment.

Although the youngest fully emerged leaves were unable fully to compensate for lost tissue, the extent of compensation clearly differed among defoliation treatments. Such findings have important implications for defoliation studies. The use of herbivores in manipulative experiments can be a logistical nightmare, particularly when trying to uncouple plant susceptibility from plant tolerance to herbivory (Welter 1991). Therefore, defoliation studies usually simulate feeding by clipping leaves. Most herbivory studies have simulated either timing or level of natural defoliation (Wallace *et al.* 1984; Chapman *et al.* 1990; Evans 1991; Marquis 1991; Senock *et al.* 1991; Welter 1991; Marquis 1992). Few studies have considered the

actual pattern of tissue removal by herbivores (Marshall 1989; Marquis 1991, 1992). Herbivores can remove leaf tissue in different, often species-specific patterns; for example, notches in leaves by leaf cutter bees and weevils, perforations by borers, and skeletonizing by leaf-feeding beetles (Metcalf *et al.* 1962; Pedigo 1989). We demonstrated that such differences in the pattern of defoliation can have very different effects upon photosynthetic capacity of residual tissue. In addition to simulating timing and level of defoliation, future studies which attempt to assess the impact of herbivores on plant fitness should document and mimic natural defoliation patterns. Furthermore, attempts to compare the impact of different herbivores should consider that variation in response to herbivory may be due simply to differences in the pattern of tissue removal.

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References

Belsky, A.J. (1986) Does herbivory benefit plants? A review of the evidence. *American Naturalist*, **127**, 870–892.

Chapman, D.F., Robson, M.J. & Snaydon, R.W. (1990) Short-term effects of manipulating the source: sink ratio of white clover (*Trifolium repens*) plants on export of carbon from, and morphology, developing leaves. *Physiologia Plantarum*, **80**, 262–266.

Crawley, M.J. (1989) Insect herbivores and plant population dynamics. *Annual Review of Entomology*, **34**, 531–564.

Evans, A.S. (1991) Whole-plant responses of *Brassica campestris* (Cruciferae) to altered sink–source relations. *American Journal of Botany*, **78**, 394–400.

Gedge, K.E. (1992) *Effects of herbivory on two Great Lakes beach annuals*: Cakile edentula *and* Corispermum hyssopifolium. MSc thesis, Department of Plant Science, University of Western Ontario.

Hall, I.V., Steiner, E., Threadgill, P. & Jones, R.W. (1988) The biology of Canadian weeds. 84. *Oenothera biennis* L. *Canadian Journal of Plant Science*, **68**, 163–173.

Hoogesteger, J. & Karlsson, P.S. (1992) Effects of defoliation on radial stem growth and photosynthesis in the mountain birch *Betula pubescens* ssp. *tortuosa*). *Functional Ecology*, **6**, 317–323.

Isebrands, J.G. & Larson, P.R. (1973) Anatomical changes during leaf ontogeny in *Populus deltoides*. *American Journal of Botany*, **60**, 199–208.

Kim, T.H., Ourry, A., Boucaud, J. & Lemaire, G. (1991) Changes in source–sink relationship for nitrogen during regrowth of lucerne (*Medicago sativa* L.) following removal of shoots. *Australian Journal of Plant Physiology*, **18**, 593–602.

Marquis, R.J. (1991) Physiological constraints on response by *Ostrya virginiana* (Betulaceae) to localized folivory. *Canadian Journal of Botany*, **69**, 1951–1955.

Marquis, R.J. (1992) A bite is a bite is a bite? Constraints on response to folivory in *Piper arieianum* (Piperaceae). *Ecology*, **73**, 143–152.

Marshall, D.L. (1989) Integration of response to defoliation within plants of two species of *Sesbania*. *Functional Ecology*, **3**, 207–214.

Maschinski, J. & Whitham, T.G. (1989) The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *American Naturalist*, **134**, 1–19.

Mattson, W. J, and N.D. Addy. (1975) Phytophagous insects as regulators of forest primary production. *Science*, **190**, 515–521.

McNaughton, S.J. (1983) Compensatory plant growth as a response to herbivory. *Oikos*, **40**, 329–336.

McNaughton, S.J. (1984) Physiological and ecological implications of herbivory. *Encyclopedia of Plant Physiology* (eds O. L. Lange *et al.*), pp. 657–677. Springer-Verlag, Berlin.

Metcalf, C.L., Flint, W.P. & Metcalf, R.L. (1962) *Destructive and Useful Insects*. 4th edn. McGraw-Hill, New York.

Mihaliak, C.A. & Lincoln, D.E. (1989) Plant biomass partitioning and chemical defense: response to defoliation and nitrate limitation. *Oecologia*, **80**, 122–126.

Morrow, P.A. & LaMarche, V.C. (1978) Tree ring evidence for chronic insect suppression of productivity in subalpine *Eucalyptus*. *Science*, **201**, 1244–1246.

Paige, K.N. & Whitham, T.G. (1987) Overcompensation in response to mammalian herbivory: the advantage of being eaten. *American Naturalist*, **129**, 407–416.

Pedigo, L.P. (1989) *Entomology and Pest Management*. MacMillan, New York.

Senock, R.S., Sisson, W.B. & Donart, G.B. (1991) Compensatory photosynthesis of *Sporobolus flexuosus* (Thurb.) Rydb. following simulated herbivory in the northern Chihuahuan Desert. *Botanical Gazette*, **152**, 275–281.

Strauss, S.Y. (1988) Determining the effects of herbivory using naturally damaged plants. *Ecology*, **69**, 1628–1630.

Wallace, L.L., McNaughton, S.J. & Coughenour, M.B. (1984) Compensatory photosynthetic responses of three African graminoids to different fertilization, watering and clipping regimes. *Botanical Gazette*, **145**, 151–156.

Welter, S.C. (1991) Responses of tomato to simulated and real herbivory by tobacco hornworm (Lepidoptera: Sphingidae). *Environmental Entomology*, **20**, 1537–1541.

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