Impairment of Leaf Photosynthesis After Insect Herbivory or Mechanical Injury on Common Milkweed, *Asclepias syriaca*

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ABSTRACT Insect herbivory has variable consequences on plant physiology, growth, and reproduction. In some plants, herbivory reduces photosynthetic rate (Pn) activity on remaining tissue of injured leaves. We sought to better understand the influence of leaf injury on Pn of common milkweed, *Asclepias syriaca* (Asclepiadaceae), leaves. Initially, we tested whether Pn reductions occurred after insect herbivory or mechanical injury. We also (1) examined the duration of photosynthetic recovery, (2) compared mechanical injury with insect herbivory, (3) studied the relationship between leaf Pn with leaf injury intensity, and (4) considered uninjured leaf compensatory Pn responses neighboring an injured leaf. Leaf Pn was significantly reduced on mechanically injured or insect-fed leaves in all reported experiments except one, so some factor(s) (cardiac glycoside induction, reproductive investment, and water stress) likely interacts with leaf injury to influence whether Pn impairment occurs. Milkweed tussock moth larval herbivory, *Euchaetes egle* L. (Arctiidae), impaired leaf Pn more severely than mechanical injury in one experiment. Duration of Pn impairment lasted >5 d to indicate high leaf Pn sensitivity to injury, but Pn recovery occurred within 13 d in one experiment. The degree of Pn reduction was more severe from *E. egle* herbivory than similar levels of mechanical tissue removal. Negative linear relationships characterized leaf Pn with percentage tissue loss from single *E. egle*–fed leaves and mechanically injured leaves and suggested that the signal to trigger leaf Pn impairment on remaining tissue of an injured leaf was amplified by additional tissue loss. Finally, neighboring uninjured leaves to an *E. egle*–fed leaf had a small (≈10%) degree of compensatory Pn to partly offset tissue loss and injured leaf Pn impairment.

KEY WORDS gas exchange, plant–insect interaction, compensatory photosynthesis, defoliation, folivory

Plants experience a variety of abiotic and biotic stresses that prevent optimal growth. One biotic stress is herbivory, which removes and/or injures plant tissues. Across and within plant species, response to herbivory injury is highly variable, ranging from overcompensation, to no net damage, and to net harm (McNaughton 1983, Maschinski and Whitham 1989, Welter 1989, Belsky et al. 1993, Peterson and Higley 1993, Coley and Barone 1996, Paige 1999, Delaney and Macedo 2001). This variation is partly because of a variety of compensatory mechanisms: delayed leaf senescence, assimilate reallocation, leaf overproduction, changes in canopy architecture, and compensatory photosynthesis (Chapin 1991, Peterson and Higley 1993, 2001, Trumble et al. 1993). However, in entomological studies, the interest is often on how insect herbivory affects plant yield, whereas the physiological mechanisms by which injury leads to subsequent plant damage is often treated as a black box (Higley et al. 1993, Peterson and Higley 1993, 2001). Thus, more needs to be understood about the mechanisms by which insect herbivory physically and physiologically alter plants that lead to changes in plant growth, yield, and fitness.

Some variation in plant responses to insect folivory relates to differences in spatial scale considered, ranging from complete whole plant defoliation to incomplete insect consumption on a single leaf (Welter 1989). Because photosynthetic leaf tissue is lost after folivory, changes in plant growth and reproduction depend on herbivory intensity and plant compensatory mechanisms (Trumble et al. 1993). If the deleterious effects of a stress like folivory cannot be contained within an injured leaf, larger-scale mechanisms become necessary for a plant to tolerate or partially compensate for injury (Welter 1989, Dickson and Isebrands 1991, Peterson and Higley 1993, Trumble et al. 1993). Thus, leaf level responses are an important component of overall plant response to stress (Dickson and Isebrands 1991).

Net photosynthetic rate (Pn) is one useful, rapidly obtained plant primary physiological assay for study-

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ing how insect herbivory like partial consumption of individual leaves affects injured or nearby uninjured leaf physiology (Welter 1989, Peterson and Higley 1993, 2001, Neves et al. 2006). Across plant species, photosynthetic activity is fairly consistently impaired by some types of herbivory such as aphid phloem feeding, stem boring, and leaf mining (Welter 1989). However, folivorous herbivory has variable effects where leaf photosynthetic rate (Pn) can increase, not change, or decrease on injured or uninjured leaves across plant species (Welter 1989, Meyer and Whitlow 1992; Peterson and Higley 1993, Meyer 1998a, b, Oleksyn et al. 1998, Peterson et al. 1998, 2004, Zangerl et al. 2002, Mercader and Isaacs 2003, Delaney and Higley 2006, Tang et al. 2006). Peterson and Higley (1993, 2001) argued that much apparent variation in leaf Pn responses to injury exists because of failure to explicitly separate extrinsic (external environmental) from intrinsic (metabolic) plant changes after herbivory; if physiological mechanisms underlying changes in photosynthetic response to herbivory can be understood, this may allow explanations about variation in leaf and whole plant responses to insect defoliation. Also, studies can examine other environmental factors that interact with herbivory to influence plant physiology, growth, and fitness (Higley et al. 1993, Delaney and Macedo 2001).

Common milkweed, *Asclepias syriaca* (Asclepiadaceae), is a highly clonal species (Malcolm 1995). We chose to study *A. syriaca* because of investment in constitutive (Malcolm 1995) and induced (Malcolm and Zalucki 1996) cardiac glycoside defense compounds. It has been hypothesized that a trade-off exists between chemical defense investment with photosynthesis (Baldwin and Ohnmeiss 1994, Zangerl et al. 2002, Kessler et al. 2004). One prediction from this hypothesis would be that photosynthetic impairment would be expected to occur on leaves where chemical defenses have been breached. As such, we needed to study Pn impairment on injured *A. syriaca* leaves. Here, we present results of 12 *A. syriaca* experiments to examine whether Pn reductions occurred after partial leaf tissue removal from mechanical injury and multiple insect herbivores. Second, we examined the duration of Pn impairment on injured leaves, because the duration of Pn impairment is a temporal component of leaf injury severity. Third, we compared the severity of Pn impairment from similar amounts of tissue loss between mechanical injury and insect herbivory and between different insect herbivores. Fourth, we defined the shape of the relationship between individual leaf injury intensity (percentage of tissue removal) with leaf Pn on an injured leaf's remaining tissue. Finally, we studied whether compensatory Pn occurs on uninjured leaves near to an *Euchaetes egle*–fed leaf, to determine whether this compensatory mechanism can partly offset the impact of tissue loss and Pn impairment on an injured leaf. Thus, the experiments reported consider different aspects of how partial tissue consumption on single leaves affects the degree of Pn impairment on remaining tissue of an injured *A. syriaca* leaf.

Materials and Methods

Study Sites. There were two locations at which we conducted our *A. syriaca* experiments. Eight of 12 experiments were conducted in the tall-grass portion of Nine-Mile Prairie (71 ha), located 15 km north northwest from the downtown of the city of Lincoln in Lancaster County, NE, and managed by the University of Nebraska-Lincoln. This site has tall bluestem grass as a dominant species, sumac was spreading throughout the site, and *A. syriaca* was located in large patches throughout Nine-Mile Prairie. The tall grass portion of Nine-Mile Prairie was maintained on a 3-yr burn cycle. Herbivores observed on *A. syriaca* at Nine-Prairie were adult milkweed beetles (*Tetroapes tetrophthalmus* Forster 1771; Cerambycidae), adult milkweed stem weevils (*Rhyssomatus lineaticollis* Say; Curculionidae), and unexpected cycnia moth larvae (*Cycnia inopinatus* H. Edwards 1882; Arctiidae).

The second study site location, where the other four experiments were conducted, was a 2-ha tall-grass prairie site that was located on the University of Nebraska-Lincoln's agricultural campus (UNL). This university site predominately contained tall bluestem grasses and *A. syriaca* throughout and was maintained on an annual burn cycle. The most common *A. syriaca* herbivores at the UNL east campus site were *T. tetrophthalmus* adults, although adult *R. lineaticolis* and monarch butterfly larvae (*Danaus plexippus* L; Nymphalidae) were also occasionally found here (unpublished data). The most common herbivores available for use in our *A. syriaca* herbivory studies (Delaney 2003) were milkweed tiger moth larvae (*Euchaetes egle* Drury 1773; Arctiidae), which were not collected from either research site. Instead, *E. egle* larvae were consistently found on *A. syriaca* surrounding a private home (P. Taylor, unpublished data).

For experiments involving insect herbivores, mesh cages were used to restrict the herbivores to a single *A. syriaca* leaf, and each cage was sealed with tape at the leaf's petiole. Insects were always removed after 24 h, while initial leaf postinjury photosynthetic measurements were collected either 24 or 48 h after herbivores were introduced. Postinjury photosynthetic measures were collected at the same time of day that herbivores were placed onto leaves for an experiment (and same time of day of any preinjury baseline measurements). Additional postinjury durations were collected in some experiments to examine the duration of photosynthetic reductions. Preinjury baseline photosynthetic measurements were collected in four experiments and are indicated in the details for each of those experiments in the next section. Uninjured control leaves also received a mesh cage for 24 h to control for any cage effects in experiments where insect herbivores were involved, whereas uncaged leaves were used when a mechanical leaf injury was included. Control leaves always occurred on different *A. syriaca* stems than those with any insect or mechanical herbivory treatments and at the same leaf pair height as corresponding injured leaves (third–fifth) to offer leaves with high photosynthetic activity and to stan-

dardize leaf position. All experiments were conducted during the summer, where air temperatures >30°C. Experiments are described in the order in which they were conducted, and a different group of *A. syriaca* stems were used in each experiment.

Individual Experiment Details. Experiment 1 began on 28 July 1994 and was conducted at Nine-Mile Prairie. There were four leaf treatments: mesh caged uninjured leaves ($n = 5$), uncaged uninjured leaves ($n = 5$), uncaged mechanically injured (25–50% tissue removal) leaves ($n = 5$), and caged *E. egle* larval-fed (25–50% tissue removal) leaves ($n = 4$). Multiple (3–5) fourth or fifth instar *E. egle* larvae were placed on a treatment leaf. Mechanical injury was imposed on a leaf by using scissors. Since mechanical leaf injury didn't require a mesh cage, uncaged leaves served as the appropriate uninjured controls for comparison. Only one leaf treatment occurred on a single *A. syriaca* stem, so 19 stems were used in experiment 1 and treatments were completely randomized among stems. Leaf Pn measurements were collected from all leaf treatments at 1d postinjury, where one-way analysis of variance (ANOVA) with leaf treatment as the fixed factor was used to analyze the data. Uninjured ($n = 4$; one leaf was unavailable) and *E. egle*-fed leaf ($n = 4$) Pn was also measured at 5d postinjury, so a two-sample T-test was used to analyze this data.

Experiment 1 above and experiments 2 and 3 below all used a closed system infrared gas analyzer (LI-6200; Li-Cor, Lincoln, NE) to collect photosynthetic measurements. With the LI-6200, two to four Pn (μmol $CO_2/m^2/s$) measurements per leaf were collected within 120 s, and the values were averaged to yield a single value for each leaf for data analysis. Measurements were collected from leaves in a standard 1-liter chamber where tissue from only part of a leaf filled the chamber so that comparisons among injured and uninjured leaves were made on the same per leaf area basis. Measurements were collected from 1200 to 1400 hours when photosynthetically photon flux densities (PPFD) levels were >1,400 μmol/m^2/s (measured with a LI-190 quantum sensor; Li-Cor) so that all *A. syriaca* leaves were light saturated (Delaney 2003).

Experiment 2 began on 30 July 1994 at Nine-Mile Prairie. In this *A. syriaca* experiment, there were five leaf treatments: caged uninjured leaves ($n = 5$), one caged *E. egle* fourth- or fifth-instar larva on leaves ($n = 4$), two caged *E. egle* fourth- or fifth-instar larvae on leaves ($n = 4$), three caged *E. egle* fourth- or fifth-instar larvae on leaves ($n = 4$), or one caged *T. tetrophthalmus* adult on leaves ($n = 4$), with cages removed after 24 h. After mesh cages were removed, 1-d postinjury, leaf Pn measurements were collected (note: one *T. tetrophthalmus* leaf and one *E. egle* leaf received no feeding injury and therefore were included as uninjured leaves in the analysis). To measure the percentage leaf tissue consumption by *E. egle* or *T. tetrophthalmus*, a preinjury tracing was made of each leaf onto paper in the field, and lost tissue was also traced onto the leaf tracing. Back at the laboratory, the tracing for each leaf was cut out and run through a leaf area meter (LI-3100; Li-Cor) to measure leaf area in square centimeter. Then the lost tissue tracings were cut out of leaves, and postinjury leaf area was also measured. The percentage leaf tissue removal from each leaf was determined by $[(\text{Area}_{\text{initial}} - \text{Area}_{\text{final}})/\text{Area}_{\text{initial}}]$ to standardize the amount of leaf tissue removed for leaves of different areas. Fourteen herbivore-fed and seven uninjured leaves had 1-d postinjury Pn measurements collected. The data were linear and leaf Pn response to tissue loss intensity had no threshold so least squares linear regression tested for a significant negative linear relationship between leaf Pn and percentage of single leaf tissue loss.

Experiment 3 started on 31 July 1994 at Nine-Mile Prairie. Pn measurements were collected from the same treatment leaves at 2, 4, and 13 d postinjury for repeated measures. Because of temporal overlap with experiments 1 and 2, 1-d postinjury measurements could not also be collected in experiment 3. There were three leaf treatments: caged uninjured leaves ($n = 4$), caged *T. tetrophthalmus*–fed (5–10% tissue removal) leaves ($n = 4$), and caged *E. egle*–fed (25–50% tissue removal) leaves ($n = 9$ at 2 and 4 d postinjury; $n = 7$ at 13 d postinjury). Again, three to five fourth- or fifth-instar *E. egle* larvae were placed on a leaf and caged, whereas three *T. tetrophthalmus* adults (more led to overcrowding; R.K.D. Peterson, unpublished data) were placed onto a leaf and caged. Because of the high adult *T. tetrophthalmus* mobility, only four leaves were able to be set-up for this treatment. Much greater tissue loss occurred from *E. egle* than *T. tetrophthalmus* herbivory, so experiment 3 compared moderate versus low levels of tissue loss rather than a direct leaf Pn herbivory comparison between these two insects. One leaf treatment occurred per *A. syriaca* stem, so leaf treatments were completely randomized across 17 *A. syriaca* stems. Repeated-measures analysis of variance (ANOVA) analyzed whether leaf treatments had differential effects (impairment and/or recovery) on leaf Pn over time. A one-way ANOVA with leaf treatment as a fixed factor was performed on Pn data at each measurement date to determine herbivory effects on leaf Pn by both insect herbivores at each date.

For experiments 4–12, we used an open system infrared gas analyzer (LI-6400; Li-Cor) to collect Pn data. A red-blue light source in the measurement chamber shined PPFDs of 1,500–2,000 μmol/m^2/s onto measured *A. syriaca* leaves (Delaney 2003) to match ambient light intensities (one value set for each experiment). After complete CO_2 and partial H_2O removal from intake air, a CO_2 mixer set reference $[CO_2]$ to 400 μmol CO_2/mol air in air heading to the measurement chamber. No temperature control was used in any experiments. One Pn measurement was needed per leaf with the LI-6400 because of the short duration to reach high CO_2 and H_2O stability.

Experiment 4 started on 12 June 1997 at Nine-Mile Prairie. There were two leaf treatments, uncaged uninjured leaves ($n = 5$) and uncaged mechanically injured leaves ($n = 5$), and leaf injury was imposed at 1000 hours. Leaf mechanical injury was imposed by scissors as 50% of a single leaf's area was removed

(visual estimation). Photosynthetic measurements were collected from 1200 to 1300 hours at 2 and 4 d postinjury. One treatment leaf was measured from each *A. syriaca* stem, so 10 stems were measured in this experiment. A completely randomized design was used. Repeated-measures ANOVA tested whether the duration of Pn impairment from mechanical injury lasted 4 d or whether Pn recovery occurred on injured leaves. Two-sample *t*-tests compared Pn of uninjured control and mechanically injured leaves at 2 and 4 d postinjury.

Experiment 5 began on 27 August 1998 at Nine-Mile Prairie. Multiple fourth- or fifth-instar *E. egle* larvae were placed onto leaves starting at 1200 hours so 24 h postinjury leaf Pn measurements also started at 1200 hours. Each leaf occurred on a separate *A. syriaca* stem, and there were 20 stems in this experiment. A LI-3100 leaf area meter was used to measure a pre- and postinjury paper tracing for each leaf to calculate percentage leaf tissue loss (same as experiment 2). After leaf cage removal from the first leaf, 1-d postinjury leaf Pn measurements began at 1200 hours. Least squares linear regression was used to test whether a significant negative linear relationship existed between leaf Pn and percentage leaf tissue loss.

Experiment 6 started on 5 July 2000 at Nine-Mile Prairie. Focal leaf height, whether a focal leaf received *E. egle* herbivory, and leaf position relative to a focal leaf were three experimental factors with *A. syriaca.* A focal leaf referred to the leaf position where *E. egle* would be placed and the same leaf pair height for the corresponding uninjured control leaf on a different *A. syriaca* stem. High height focal leaves were chosen from third to fifth leaf pairs, whereas medium height focal leaves were chosen from 8th to 10th leaf pairs. Five fourth- or fifth-instar *E. egle* larvae were caged on each high height and medium height focal *E. egle* herbivory treatment leaf (visual estimation: 25–50% tissue removal). Corresponding high height and medium height uninjured focal leaves were caged. An *E. egle*–fed focal leaf at high and medium stem heights occurred on separate *A. syriaca* stems, whereas uninjured focal leaves for both heights were measured on a third stem, so a group of three stems formed a block and involved a split plot design. Whole plants served as main plots while focal leaf injury × leaf position was the split plot; this allowed appropriate error terms to be used to test for the effects of the three experimental factors on leaf Pn. Leaf position referred to a focal injured or uninjured leaf and neighboring uninjured leaves: the opposite uninjured leaf in the same leaf pair as its focal leaf, an uninjured leaf from one leaf pair above its focal leaf, and an uninjured leaf from one leaf pair below its focal leaf. Leaf Pn was collected from a focal *E. egle*–fed or uninjured leaf and the three neighboring uninjured leaves. Seven fully crossed blocks were set-up with focal uninjured and *E. egle* leaves in mesh cages, but *E. egle* larvae did not feed on two focal medium height leaves. As a result, sample sizes as follows: were medium height focal uninjured leaves ($n = 7$), high height focal uninjured leaves ($n = 7$), high height focal *E. egle*-fed leaves ($n = 7$), and medium height focal *E. egle*–fed leaves ($n = 5$). A total of 14 focal uninjured leaves and 42 uninjured neighboring leaves from seven *A. syriaca* stems, 12 focal *E. egle*–fed leaves, and 36 uninjured neighboring leaves had both baseline and 2-d postinjury measurements collected (104 leaves total on 19 stems). Baseline Pn measurements were collected to serve as a covariate to account for any preinjury variation in leaf Pn across treatments; both baseline and 2-d postinjury leaf Pn were collected from 1300 to 1500 hours. A split-plot mixed model analysis of covariance (ANCOVA) tested Pn data for compensatory Pn on uninjured leaves adjacent to an *E. egle*–fed leaf and whether leaf height influenced the degree of Pn impairment on *E. egle*–fed leaves or the degree of compensatory Pn on nearby uninjured leaves.

Experiment 7 began on 12 August 2000 at the UNL East campus tall grass prairie site. This experiment had three leaf treatments: caged uninjured leaves ($n = 5$), caged *E. egle*–fed leaves ($n = 5$), and caged *D. plexippus*–fed leaves ($n = 5$). Two fourth- or one fifth-instar *D. plexippus* larva(e) or 10 *E. egle* larvae were placed onto a leaf with a mesh cage for 24 h. The different numbers of larvae between the two species were placed on leaves to attempt to acquire similar ranges of single leaf tissue loss (30–90%). The ranges of percentage tissue removal were similar between the two herbivore species in that a couple of leaves had low (30–40%) tissue removal from each species, a couple of leaves had high (70–90%) tissue removal from each species, and one leaf had medium (50–60%) tissue removal by each species. This allowed experiment 7 to directly test for differential leaf Pn effects from two milkweed specialist herbivores. However, note that midrib cutting injury was observed on most *D. plexippus*–fed leaves but no *E. egle*–fed leaves at 2-d postinjury; this was determined by looking underneath treatment leaves for cutting injury on the midrib. Preinjury baseline Pn was collected from 1400 to 1500 hours, and 2-d postinjury leaf Pn measures were collected at the same time of day. One treatment leaf occurred on a single *A. syriaca* stem, and groups of three stems formed blocks so that 15 stems were used in experiment 7. Block was a nonsignificant random factor in data analysis (unpublished data), so final models did not include block. Repeated-measures ANOVA tested for differences in baseline and 2-d postinjury leaf Pn of injured and uninjured leaves. A one-way ANOVA tested whether any baseline preinjury leaf Pn differences occurred among treatment leaves. A one-way ANCOVA with preinjury baseline Pn as a covariate tested whether 2-d postinjury leaf Pn was significantly reduced on herbivore-fed compared with control uninjured leaves.

Experiment 8 began on 16 August 2000 at the UNL East campus tall grass prairie site, with a different group of *A. syriaca* stems than used in experiment 7. There were four leaf treatments: caged uninjured leaves ($n = 9$), caged *E. egle*-fed leaves ($n = 9$), caged *D. plexippus*-fed leaves ($n = 5$), and caged salt marsh tiger moth (*Estigmene acrea* Drury 1773; Arctiidae) larval-fed leaves ($n = 4$). Either 1 fifth-instar *D. plexip-*

pus larva, 1 fifth-instar *Estigmene acrea* larva, or 10 *E. egle* larvae were placed onto a leaf. Similar ranges of single leaf tissue loss were visually estimated by feeding from each herbivore species (30–80%). Baseline Pn measures were collected from 1300 to 1400 hours on 16 August, and 1-d postinjury leaf Pn was collected at the same time of day. Only two *Estigmene acrea* (a generalist herbivore collected feeding on *A. syriaca*) larvae were available on 16 August. Thus, two more leaves were set up for *E. acrea* herbviory on 17 August for baseline Pn measurements, and 1-d postinjury leaf Pn was measured on 18 August. The 2-d were similar in having bright sunlight intensity (>1,500 μmol/m^2/s) based on quantum sensor readings on the LI-6400 measurement chamber head and high air temperatures (>30°C) based on thermocouple readings in the LI-6400 measurement chamber (K.J.D., unpublished data). One treatment leaf occurred on a single *A. syriaca* stem, so 27 stems were randomized across treatments in experiment 8. Repeated-measures ANOVA tested for differences in baseline and 1-d postinjury leaf Pn of injured and uninjured leaves. A one-way ANOVA tested whether any baseline preinjury leaf Pn differences occurred among treatment leaves. A one-way ANCOVA with preinjury baseline Pn as a covariate tested whether 1-d postinjury leaf Pn was significantly reduced on herbivore-fed compared with control uninjured leaves.

Experiment 9 began on 15 June 2001 at Nine-Mile Prairie. This experiment had two leaf treatments: caged uninjured leaves ($n = 16$) and caged *D. plexippus* larval-fed leaves ($n = 16$). Either two third/fourth-instar larvae or one fifth-instar *D. plexippus* larva were/was placed on each herbivory treatment leaf. This experiment tested whether *D. plexippus* herbivory at an earlier time in *A. syriaca's* growing season would cause significant Pn impairment on remaining injured leaf tissue compared with uninjured control leaf Pn, because our other early season study (experiment 4) only tested the effects of mechanical injury on leaf Pn. Each leaf treatment occurred on an *A. syriaca* stem, so treatment leaves were completely randomized among 32 stems in experiment 9. Repeated-measures ANOVA tested whether differences in baseline and 1-d postinjury leaf Pn occurred on injured and uninjured leaves. A two-sample *t*-test was used to compare baseline Pn of control and *D. plexippus* assigned leaves. An ANCOVA with baseline Pn as the covariate and leaf treatment as the fixed factor tested whether *D. plexippus* herbivory significantly reduced 1-d postinjury leaf Pn compared with uninjured control leaves.

Experiment 10 began on 1 August 2001 at Nine-Mile Prairie. There were caged uninjured control leaves ($n = 8$), 2 *E. egle* larvae on leaves ($n = 3$), 4 *E. egle* larvae on leaves ($n = 3$), 5 *E. egle* larvae on leaves ($n = 3$), 8 *E. egle* larvae on leaves ($n = 4$), and 10 *E. egle* larvae on leaves ($n = 4$) to obtain a wide range of percentage leaf tissue removal from single *A. syriaca* leaves. Baseline and 1-d postinjury leaf Pn measurements were collected from 1400 to 1530 hours. The percentage of single leaf tissue removal from *E. egle* herbivory was visually estimated (nearest 5%). There was one leaf per stem, so 25 *A. syriaca* stems were in experiment 10. No signs of nonlinearity or threshold responses were observed in the data, so least squares linear regression tested whether a significantly negative linear relationship existed between 1-d postinjury leaf Pn with percentage single leaf tissue loss.

Experiment 11 began on 6 June 2002, experiment 12 began on 25 June 2002, and both were conducted at the UNL tall grass prairie site. The two experiments each had three leaf treatments with uncaged uninjured control leaves, leaves with 25% mechanical tissue removal, and leaves with 75% mechanical tissue removal; 1-d postinjury Pn measurements were collected starting at 1300 hours. The experiments differed in sample sizes; experiment 11 had $n = 6$ for each leaf treatment for a total of 18 leaves measured, whereas experiment 12 had $n = 5$ for each leaf treatment for a total of 15 leaves measured. Both experiments had one treatment leaf per *A. syriaca* stem, and treatments were randomized across stems, so there were 18 stems in experiment 11 and 15 stems in experiment 12. Least squares linear regression tested whether a significantly negative linear relationship existed between 1-d postinjury leaf Pn with percentage single leaf tissue loss for both experiments 11 and 12.

Results

Herbivory Effects on *A. syriaca* Leaf Pn. In experiment 1, caged and uncaged uninjured control leaves had significantly higher Pn at 1-d postinjury compared with mechanically injured leaves or *E. egle*–fed leaves ($F_{3,15} = 39$, $P < 0.0005$; Fig. 1). *E. egle*–fed leaves had significantly lower 1-d postinjury leaf Pn than mechanically injured leaves (Fig. 1), even though both treatments involved similar amounts of leaf injury. Caged and uncaged uninjured mean leaf Pn's were not significantly different from each other (Fig. 1). At 5-d postinjury, *E. egle*–fed leaves (7.0 ± 3.05, $n = 4$) still had a lower mean Pn than caged uninjured leaves (17.9 ± 1.12, $n = 4$) in experiment 1 (not shown in Fig. 1) based on the results of an unequal variance two sample *t*-test ($T_{3df} = 3.35$, $P = 0.03$).

In experiment 4, a significant repeated-measures ANOVA injury term ($F_{1,7} = 54$, $P < 0.0005$) showed early season Pn impairment after mechanical injury was imposed on leaves on flowering plants compared with uninjured control leaves (Fig. 2). The nonsignificant date ($F_{1,7} = 1.9$, $P > 0.20$) and date × injury ($F_{1,7} = 2.1$, $P = 0.20$) terms indicated a similar degree of injured leaf Pn impairment. Two-sample *t*-tests confirmed this at 2-h postinjury ($T_{8df} = 4.79$, $P = 0.001$) and later at 4-d postinjury ($T_{7df} = 3.56$, $P = 0.009$) in experiment 4 (Fig. 2).

In experiment 9, repeated-measures ANOVA showed a significant effect of early season *D. plexippus* larval herbivory ($F_{1,30} = 7.4$, $P = 0.01$) on injured leaf Pn compared with uninjured control leaves of flowering plants (Fig. 3). There was no significant difference in baseline leaf Pn using a two-sample *t*-test ($T_{30df} = 1.11$, $P > 0.20$) between uninjured control

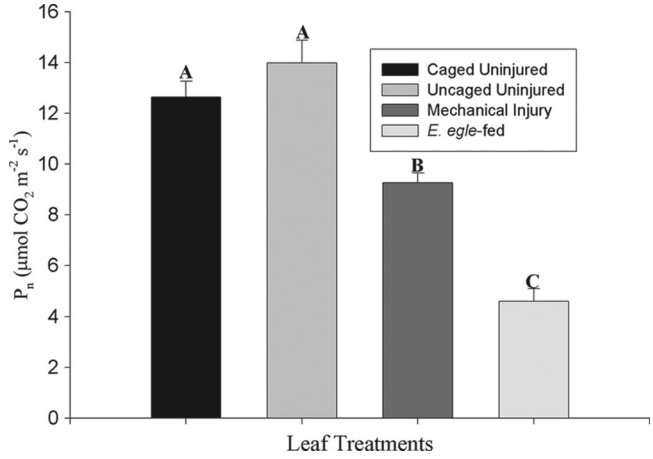

Fig. 1. Mean *A. syriaca* leaf Pn response (±SE) at 1-d postinjury for experiment 1. Treatments consist of caged control (no injury), uncaged control (no injury), uncaged mechanically injured (25–50% tissue loss), and caged *E. egle*–fed leaves (25–50% tissue loss). Treatments with the same letter were not statistically distinguishable by an LSD post hoc test.

($n = 16$) and the *D. plexippus*–fed ($n = 16$) leaves. The date term in this repeated-measures ANOVA was nonsignificant ($F_{1,30} = 0.3$, $P > 0.5$), so overall leaf Pn was similar between baseline and 1-d postinjury. However, a significant date × injury term ($F_{1,30} = 8.8$, $P = 0.006$) in experiment 9 helped to show that 1-d postinjury uninjured leaf Pn increased, whereas *D. plexippus*–fed leaf Pn decreased (Fig. 3). An ANCOVA showed baseline Pn was a highly significant covariate ($F_{1,29} = 12$, $P = 0.002$), and 1-d postinjury leaf Pn was significantly lower on *D. plexippus*–fed leaves than uninjured control leaves ($F_{1,29} = 7.8$, $P = 0.009$).

In experiment 3, a repeated-measures ANOVA showed that uninjured caged control leaves and *T. tetrophthalmus*–fed leaves were significantly different than leaves with *E. egle* herbivory (injury: $F_{2,12} = 7.9$, $P < 0.01$; date; $F_{2,24} = 77$, $P < 0.0005$; injury × date: $F_{4,24} = 5.4$, $P < 0.01$). There was no difference in leaf Pn between uninjured leaves and *T. tetrophthalmus*–fed leaves at 2-, 4-, and 13-d postinjury in experiment 3 (Fig. 4). Control uninjured *A. syriaca* leaf Pn and *T. tetrophthalmus*–fed leaf Pn were both significantly higher than *E. egle*-fed leaf Pn at 2-d postinjury ($F_{2,14} = 25$, $P < 0.0005$), and control uninjured leaves had significantly higher leaf Pn than *E. egle*–fed leaves at 4-d postinjury ($F_{2,14} = 6.0$, $P = 0.01$; Fig. 4). No differences were detected between the three leaf treatments at 13-d postinjury ($F_{2,12} = 0.4$, $P > 0.5$).

In experiment 7 with *E. egle*–fed and *D. plexippus*–fed leaf treatments, the injury treatment term was not

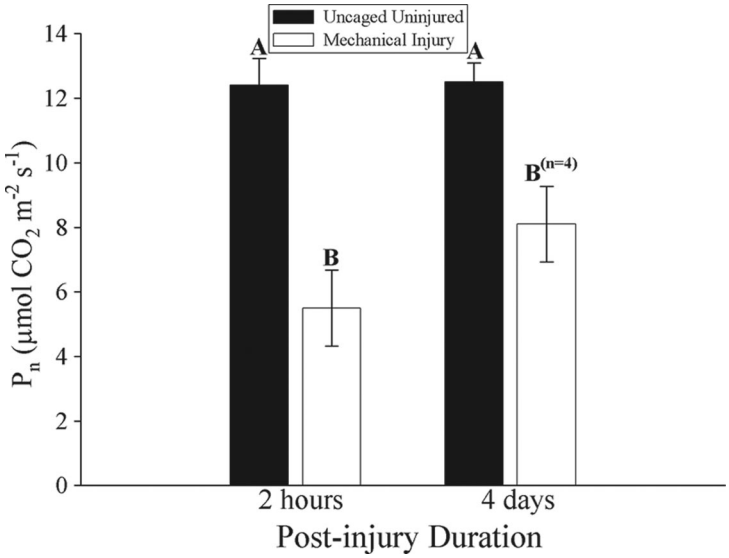

Fig. 2. Mean leaf Pn response (±SE) at 2 h and 4 d postinjury for uncaged uninjured and uncaged mechanically injured (25–50% tissue loss) leaves in experiment 4. Treatments with the same letter were not statistically distinguishable by an LSD post hoc test within each date.

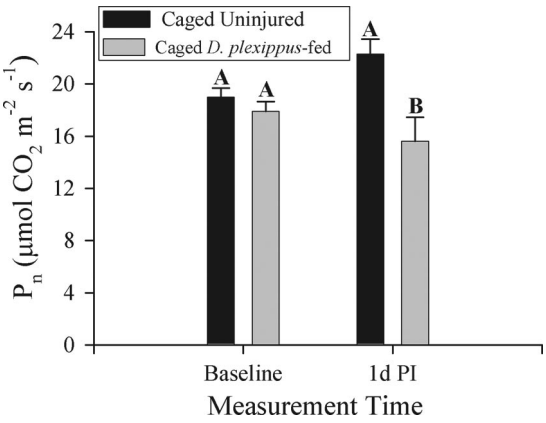

Fig. 3. Mean leaf Pn response (±SE) is shown for preinjury leaf Pn and 1-d postinjury leaf Pn for caged control uninjured and caged *D. plexippus*–fed leaves in experiment 9. Treatments with the same letter were not statistically distinguishable by an LSD post hoc test for each date.

significant from repeated-measures ANOVA ($F_{2,12}$ = 2.7, P = 0.11). The significant date ($F_{1,12}$ = 277, P < 0.0001) term reflected lower Pn for all treatments at 2-d postinjury compared with baseline values (Fig. 5A). The significant date × injury ($F_{2,12}$ = 18, P < 0.0005) term showed that significantly greater 2-d postinjury Pn reductions occurred in the two herbivore leaf injury treatments than for control uninjured leaves in experiment 7 (Fig. 5A). No differences in baseline leaf Pn occurred between control and herbivory leaf treatments ($F_{2,12}$ = 2.2, P = 0.15). ANCOVA at 2-d postinjury showed that control uninjured leaf Pn > *E. egle*–fed leaf Pn > *D. plexippus*–fed leaf Pn (injury: $F_{2,11}$ = 14, P = 0.001; covariate: $F_{1,11}$ = 5.2, P = 0.04; Fig. 5A).

Experiment 8 was performed only 4 d later than experiment 7; injury ($F_{3,23}$ = 0.8, P > 0.50) and date × injury terms ($F_{3,23}$ = 1.4, P > 0.25) from repeated-measures ANOVA were not significant. These results indicated that no statistically significant Pn reductions occurred after herbivory from *E. egle*, *D. plexippus*, or *Estigmene acrea* larvae (Fig. 5B). The date term was significant ($F_{1,23}$ = 31, P < 0.0005) in experiment 8 to indicate that Pn measures of all leaf treatments at 1-d postinjury were lower than baseline values (Fig. 5B). When leaves injured by all three herbivore species were pooled to compare herbivore-fed leaves with uninjured leaves using ANCOVA for 1-d postinjury leaf Pn data, herbivory did not significantly reduce leaf Pn ($F_{1,24}$ = 2.5, P = 0.12); baseline Pn was a significant covariate ($F_{1,24}$ = 10, P = 0.004).

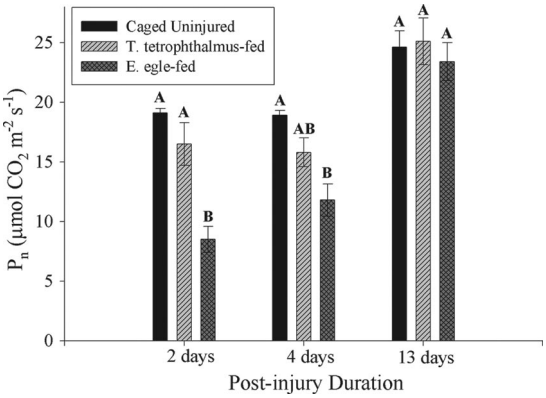

Fig. 4. Mean leaf Pn response (±SE) taken at 2-, 4-, and 13-d postinjury for experiment 3. Treatments include caged uninjured control (no injury), caged *T. tetrophthalmus*–fed (5–10% tissue loss), and caged *E. egle*–fed leaves (25–50% tissue loss). Treatments with the same letter were not statistically distinguishable by an LSD post hoc test for each date.

Intensity of Leaf Injury and Leaf Pn Responses. Significant negative linear relationships were shown between *E. egle*–fed leaf injury intensity and leaf Pn based on least squares linear regression for experiments 2 ($F_{1,19}$ = 29, r^2 = 0.59; P < 0.0005; Fig. 6A), 5 ($F_{1,18}$ = 5.2, r^2 = 0.18; P = 0.03; Fig. 6B), and 10 ($F_{1,24}$ = 14, r^2 = 0.35; P = 0.001; Fig. 6C). A marginally significant negative linear relationship occurred between mechanical leaf injury intensity with leaf Pn in experiment 11 ($F_{1,16}$ = 3.4, r^2 = 0.17; P = 0.085; Fig. 6D) and was nonsignificant in experiment 12 ($F_{1,13}$ = 2.5, r^2 = 0.16; P = 0.14; Fig. 6E). Although the amount of tissue removal by *E. egle* herbivory influenced the degree of Pn reduction in experiments 2, 5, and 10, its importance in accounting for variation in leaf Pn differed between experiments (from 18 to 59% of variance) and the slopes (±SE) varied in response to injury intensity between the three *E. egle*–fed leaf lines (−0.147 ± 0.027, −0.122 ± 0.053, and −0.064 ± 0.017). Although the mechanical injury lines' slopes (±SE) (−0.078 ± 0.042, −0.088 ± 0.056) fell within the range of slopes from *E. egle* herbivory, there was too much variability to statistically distinguish mechanical injury line slopes from zero, and variation (16 and 17% of variance) accounted by mechanical leaf injury intensity was low.

Impact of *E. egle* Feeding Injury on Pn of Focal and Neighboring Leaves. For experiment 6, leaf height ($F_{1,6df}$ = 0.2, P > 0.50), injury × leaf height ($F_{1,74df}$ = 1.3, P > 0.25), leaf position × leaf height ($F_{3,74df}$ = 0.3, P > 0.80), and injury × leaf position × leaf height ($F_{3,74df}$ = 0.3, P > 0.80) terms were not significant from a split plot mixed model ANCOVA for 2-d postinjury Pn data on late flowering plants. Terms that were significant from this analysis included injury ($F_{1,74df}$ = 5.8, P < 0.05), leaf position ($F_{3,74df}$ = 7.0, P < 0.01), injury × leaf position ($F_{3,74df}$ = 8.5, P < 0.001), and baseline Pn as the covariate ($F_{1,74df}$ = 17, P < 0.001). Both baseline and 2-d postinjury leaf Pn means (±SE) are shown for the injury × leaf position treatments (Fig. 7), because no terms involving leaf height were significant at P < 0.05. At 2-d postinjury, leaf Pn decreased the most for focal *E. egle*–fed leaves, leaf Pn decreased slightly for focal uninjured leaves and neighboring leaves to an uninjured leaf, whereas leaf Pn did not change on uninjured neighboring leaves to an *E. egle*–fed leaf (Fig. 7).

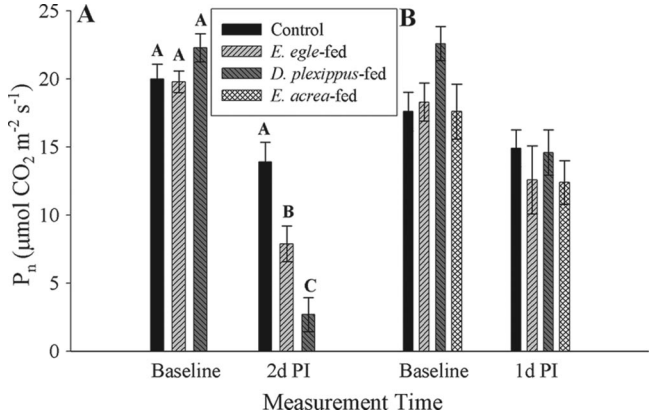

Fig. 5. Mean leaf Pn response (±SE) is shown for baseline (preinjury) leaf Pn and (A) 2-d postinjury leaf Pn for caged control uninjured ($n = 5$), caged *E. egle*–fed ($n = 5$), and caged *D. plexippus*–fed ($n = 5$; both herbivore species removed 30–90% tissue loss from individual leaves) treatments in experiment 7 and (B) 1-d postinjury leaf Pn measurements for caged control uninjured ($n = 9$), caged *E. egle*–fed ($n = 9$), caged *D. plexippus*–fed ($n = 5$), and caged *Estigmene acrea*–fed ($n = 4$) leaves (all with a range of 30–80% tissue loss from individual leaves) in experiment 8. Treatments with the same letter were not statistically distinguishable by an LSD post hoc test for each date.

Discussion

Several experiments showed that partial tissue consumption on single *A. syriaca* leaves by either of two milkweed specialist insect herbivores, *E. egle* and *D. plexippus*, as well as mechanical injury, caused photosynthetic impairment on remaining tissue of injured leaves. Across plant species, studies with partially injured leaves have detected increased Pn (Trumble et al. 1993, Meyer 1998a, Oleksyn et al. 1998) or no change in Pn (Welter 1989, Peterson and Higley 1993, Mercader and Isaacs 2003, Peterson et al. 2004, Delaney and Higley 2006), so only a subset of studies has measured decreases in injured leaf Pn (Welter 1989, Oleksyn et al. 1998, Peterson et al. 1998, Zangerl et al. 2002, Mercader and Isaacs 2003, Delaney and Higley 2006, Tang et al. 2006, Delaney 2008). Some variation in measured leaf Pn responses may be caused by measuring regrowth, injured, and/or uninjured

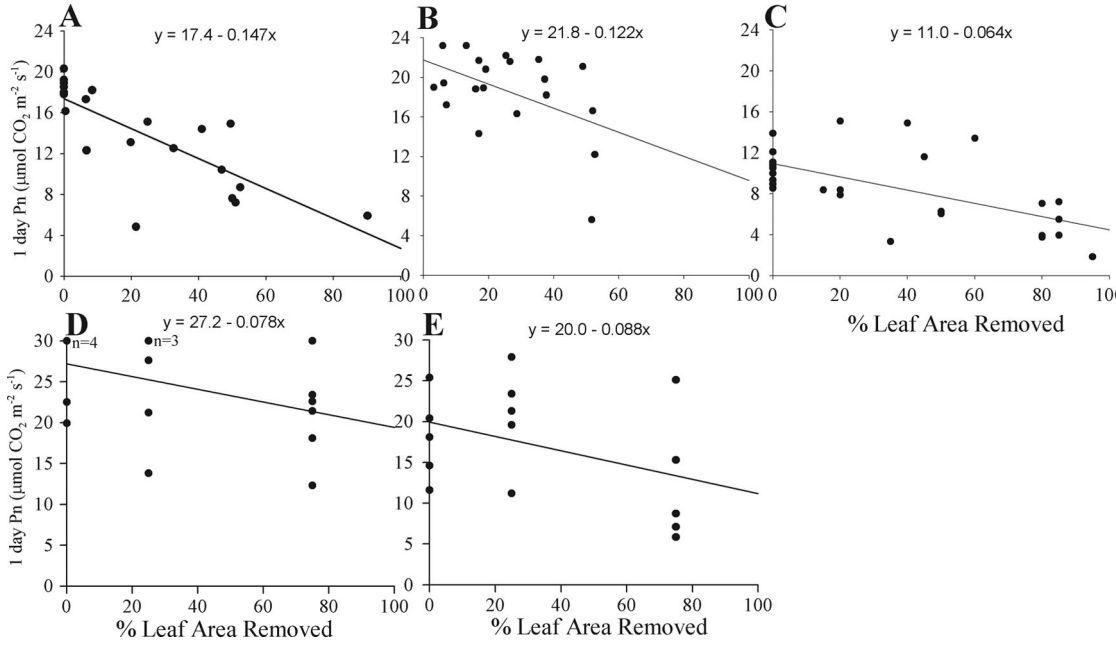

Fig. 6. Leaf Pn at 1-d postinjury is plotted across percent leaf area removal from caged uninjured and caged *E. egle*–fed leaves for (A) experiment 2, (B) experiment 5, and (C) experiment 10, and mechanical injury for (D) experiment 11 and (E) experiment 12. The best-fit line based on least squares linear regression is plotted and the equation is presented for the best-fit regression line in each graph.

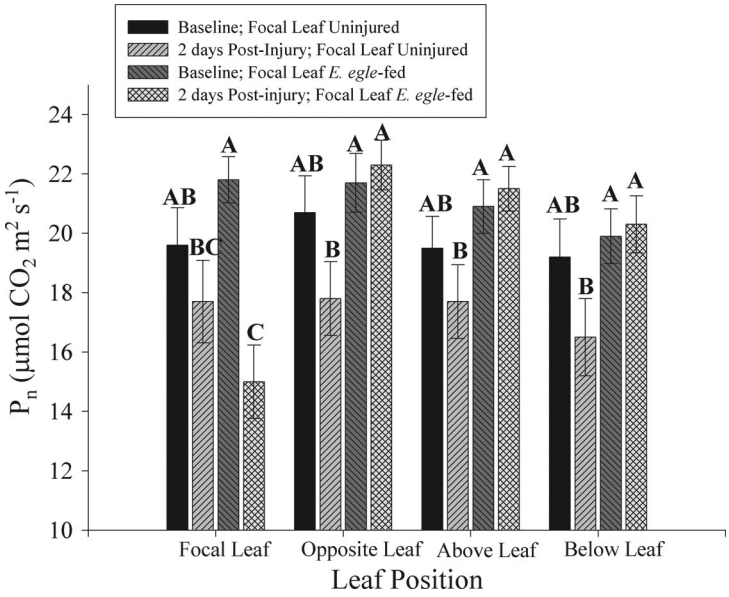

Fig. 7. Mean leaf Pn responses (±SE) are presented for the focal *E. egle*–fed and uninjured *A. syriaca* leaves (only focal leaves were caged) for baseline (preinjury) and 2-d postinjury from experiment 6. Leaf Pn is also presented for uninjured leaves (opposite leaf in same leaf pair, one leaf from the leaf pair above and one leaf from the leaf pair below) nearby either to an uninjured or *E. egle*–fed focal leaf. Treatments with the same letter were not statistically distinguishable by an LSD post hoc test.

leaves across studies (Welter 1989, Meyer and Whitlow 1992, Peterson and Higley 1993, Meyer 1998a, b, Zangerl et al. 2002, Delaney and Higley 2006). Only one leaf Pn response (increase, no change, or decrease) after leaf injury has been reported for single plant species. However, Pn impairment does not always occur after insect herbivory or mechanical injury on *A. syriaca* (experiment 8; Delaney 2003, Delaney and Higley 2006) or other milkweeds (Delaney and Higley 2006). Thus, *A. syriaca* leaf Pn response to folivory has the complication that two potential responses, reduction or no significant change in leaf Pn, require explanation.

One cause of leaf Pn impairment could be a trade-off with chemical defense investment, which motivated our *A. syriaca* studies. A resource physiological trade-off between leaf Pn impairment after herbivory with chemical defense induction has been suggested (Baldwin and Ohnmeiss 1994, Zangerl et al. 2002), and/or a hormonal regulation trade-off may occur where the same hormone stimulates chemical defense induction and photosynthetic downregulation, e.g., jasmonic acid (Kessler et al. 2004). *A. syriaca* contains constitutive cardenolides (Malcolm 1995), and positive cardenolide induction occurs after mechanical leaf injury (Malcolm and Zalucki 1996). Thus, our *A. syriaca* Pn impairment on injured leaf findings support the trade-off hypothesis between photosynthesis and chemical defenses. Another test of this hypothesis will examine whether Pn impairment only occurs when positive cardenolide induction happens with *A. syriaca* and other milkweeds. Because Pn impairment occurs in milkweed species with very low constitutive investment in cardiac glycosides (Delaney and Higley 2006), a trade-off between constitutive cardiac glycoside investment seems unlikely to explain Pn impairment after herbivory on milkweeds. Whether specialist (*E. egle* and *D. plexippus*) or generalist (e.g., *Estigmene acrea*) herbivores stimulate *A. syriaca*–positive cardenolide induction will be of interest, because induction occurs after mechanical injury (Malcolm and Zalucki 1996).

Two experiments showed Pn reductions at 4-d postinjury after mechanical injury or *E. egle* herbivory, and one experiment showed Pn reductions at 5-d postinjury after *E. egle* herbivory. Experiments 1, 4 (see Fig. 2), and 2 (Fig. 3) showed that partial leaf Pn recovery occurred on injured leaves, because the degree of Pn reductions were less at 4- to 5-d postinjury than soon after injury. Experiment 2 measured leaf Pn long enough to show that *E. egle*–fed leaf Pn was comparable to uninjured leaf Pn at 13-d postinjury and indicated complete photosynthetic recovery on remaining tissue of *E. egle*–fed leaves. Pn recovery duration influences the overall severity that insect herbivory has on leaf physiology and possibly subsequent plant growth. Other studies besides ours have reported Pn reductions lasting at least 1-wk postinjury (Meyer and Whitlow 1992, Oleksyn et al. 1998, Peterson et al. 1998, Mercader and Isaacs 2003). Shorter Pn recovery times of a few hours (Poston et al. 1976, Tartachnyk and Blanke 2003, Nabity et al. 2006) or a few days (Zangerl et al. 2002, Aldea et al. 2005) have also been reported.

With *A. syriaca*, Pn impairment from mechanical injury on a leaf was less severe than similar amounts

of leaf injury by insect herbivory in one direct comparison study, experiment 1. Also, the regression studies suggest that Pn impairment was more sensitive to leaf injury intensity from *E. egle* herbivory (experiments 2, 5, and 10) than mechanical injury (experiments 11 and 12). When similar quantities of injury occur on a plant from mechanical injury versus insect herbivory, resulting plant damage tends to be more severe from insect herbivory (Higley 1992, Marquis 1992), and more severe leaf Pn impairment (Mercader and Isaacs 2003, Delaney and Higley 2006). The temporal course of mechanical injury is usually imposed immediately on a leaf (e.g., with scissors), whereas insect herbivory occurs over the course of minutes or hours on a leaf. More recently, researchers have tried to mimic the timing of insect feeding with mechanical injury (Kessler and Baldwin 2002). Mechanical injury also lacks saliva exposure to adjacent tissue to where an insect herbivore feeds (but see Kessler and Baldwin 2002). Studies have shown that insect saliva can stimulate or inhibit plant defense responses (Korth and Dixon 1997, McCloud and Baldwin 1997, Kessler and Baldwin 2002). Thus, the activity of an insect herbivore's saliva on remaining tissue of a partially consumed leaf might help to explain more severe Pn reductions from insect feeding than mechanical leaf injury. Alternatively, different temporal patterns could result in greater water loss from injured leaf tissue by insect herbivory than mechanical injury to cause differences in Pn impairment severity. However, work by Tang et al. (2006) suggests that water loss was not the cause of *Arabidopsis thaliana* Pn reductions when detected after *Trichoplusia ni* larval herbivory.

There were two *A. syriaca* experiments where statistical differences in degree of Pn impairment were detected between two insect herbivores. With experiment 3, we suggest that the greater Pn reduction from *E. egle* than *T. tetrophthalmus* herbivory was caused by differences in amount of single leaf tissue removal rather than differences in how saliva of the two herbivores might affect *A. syriaca* leaves. The degree of Pn reduction was generally detectable at the 25–50% single leaf tissue loss range, levels typically obtained from *E. egle* herbivory. With such low leaf tissue loss (5–10%) from *T. tetrophthalmus* herbivory in experiments 2 and 3 in 1994 where injured leaf Pn could not be statistically distinguished from uninjured leaf Pn, no additional *A. syriaca* experiments studying photosynthetic impairment had adult *T. tetrophthalmus* herbivory.

Experiment 7 also showed a difference in Pn impairment between *E. egle* and *D. plexippus* herbivory that had similar ranges of tissue loss. In experiment 7, feeding by both herbivores caused significant Pn impairment, but the degree of impairment was significantly greater by *D. plexippus* than by *E. egle.* In experiment 7, most *D. plexippus*–fed leaves also had a cut midrib, whereas none of the *E. egle* leaves had a cut midrib. This was our only study where herbivore midrib cutting occurred, even though *D. plexippus*, *E. egle*, *T. tetrophthalmus*, and *R. lineaticolis* can all cut a leaf midrib on milkweeds like *A. syriaca* before feeding on nonmidrib leaf tissues (Dussourd and Eisner 1987, Dussourd and Denno 1991, Delaney and Higley 2006). Midrib injury alone impairs severely *A. syriaca* and other milkweed leaf Pn (Delaney and Higley 2006).

Another factor that influenced the severity of *A. syriaca* leaf Pn response to injury was the percentage of tissue removal from a single leaf. Our *A. syriaca* studies showed that insect herbivory intensity had a negative linear relationship with leaf Pn in experiments 2, 5, and 10 after insect herbivory, weak negative linear relationships with leaf Pn impairment after mechanical injury in experiments 11 and 12, and has been found with other plants tested (Oleksyn et al. 1998, Peterson et al. 1998, Delaney 2008, K.J.D. and L.G.H., unpublished data). In some cases, negative curvilinear relationships have also been detected where high Pn reductions occur at low injury levels (Neves et al. 2006, K.J.D. and L.G. unpublished data). A negative linear relationship between leaf Pn and injury intensity suggests that a signal(s) likely travels to remaining tissue on a partially defoliated leaf to cause Pn reductions and that the signal seems to be consistently amplified with additional tissue loss. A signal causing Pn impairment might be electrical, a hormone (e.g., jasmonic acid), or cell wall fragments detected by tissue adjacent to injured leaf regions (Hlaváčková et al. 2006).

Experiment 6 showed that *E. egle* feeding on a single *A. syriaca* leaf resulted in slight compensatory Pn of nearby uninjured leaves. However, a much larger second *A. syriaca* experiment did not detect compensatory Pn on an uninjured leaf when one half of the other leaves on the stem received either mechanical leaf tissue removal (50%) or midrib severance injury (Delaney 2003). Compensatory leaf Pn was not detected on an opposite leaf from a *D. plexippus*–fed *A. curassavica* leaf regardless of leaf injury intensity in one experiment (Delaney 2008). With *Nerium oleander* (Apocynaceae), two studies detected Pn impairment (not compensatory Pn) on an uninjured leaf when opposite (same leaf pair) from a mechanically (50%) injured leaf (Delaney 2008). For members of the milkweed plant family Asclepiadaceae and its sister family of Apocynaceae, it remains unclear to what extent compensatory photosynthesis occurs on uninjured leaves near an injured leaf, let alone specifically for common milkweed, *A. syriaca.* Compensatory photosynthesis has been reported after herbivory with several plant species. One way compensatory Pn can occur is from delayed leaf senescence of injured or uninjured leaves, where leaf Pn on injured plants does not decrease as quickly as uninjured plant leaves during normal leaf senescence (Nowak and Caldwell 1984, Haile et al. 1998, Meyer 1998b), which may explain our compensatory Pn results in experiment 6 with *A. syriaca.* Other studies have measured compensatory photosynthesis by partially defoliating plants and measuring elevated Pn on remaining uninjured leaves of partially defoliated plants (Detling et al. 1979, Nowak and Caldwell 1984, Welter 1989,

Senock et al. 1991, Hoogesteger and Karlsson 1992, Trumble et al. 1993, Syvertsen 1994).

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