

Photosynthetic Responses of Legume Species to Leaf-Mass Consumption Injury

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ABSTRACT Several studies have addressed plant primary physiological responses (gas exchange responses) to insect herbivory. However, relatively few plant species have been examined. It is unknown whether responses to leaf-mass removal by insects vary among plant families and species. Within the legumes, only alfalfa, *Medicago sativa* L., and soybean, *Glycine max* (L.) Merrill, have been examined. The goal of this study was to test the hypothesis that gas exchange responses to leaf-mass consumption in a broad range of cultivated legumes do not differ from those of soybean and alfalfa. The species and cultivars used were *M. sativa* Cimarron, *Medicago scutellata* (L.) Sava, *Medicago truncatula* Gaertner Paraggio, *Melilotus officinalis* (L.) Pallas, *Trifolium hybridum* L., and *Trifolium pratense* L. Twelve greenhouse experiments were conducted and each legume species was used in a separate experiment. Depending on the experiment, there were either two or three treatments (control, simulated insect injury, and actual insect injury). For simulated and actual insect defoliation, injury was limited to a single leaf per plant. Simulated insect injury treatments were imposed by removing leaf tissue on each leaflet with scissors. For the actual insect defoliation treatment, fall armyworm larvae, *Spodoptera frugiperda* (J.E. Smith), were used. Defoliation of single leaves on the six species resulted in similar responses. Plant gas exchange variables (photosynthesis, stomatal conductance, intercellular CO₂, and transpiration) were not significantly affected by either simulated or actual insect defoliation. Our results support the hypothesis that there is a generalized primary physiological response to leaf-mass consumption injury among the cultivated legumes. More generally, the results from this experiment support that there is a generalized plant gas exchange response to leaf-mass consumption injury.

KEY WORDS gas exchange, herbivory, alfalfa, red clover, *Medicago* spp.

MOST STUDIES OF INSECT injury and plant gas exchange have used leaf-mass consumers (defoliators). The leaf-mass consumption injury guild is characterized by removal of relatively large amounts of leaf tissue by insects (Boote 1981, Pedigo et al. 1986). Despite the relatively large number of studies that have been conducted during the past 40 yr, general models of physiological, developmental, and yield response only have been developed recently (Welter 1989, Higley et al. 1993, Peterson and Higley 2001). This was partly due to observations of highly variable photosynthetic responses as a result of defoliation injury. Many of the observations have been confounded by extrinsic (environmental) and intrinsic (physiological) factors, such as light penetration, water availability, nutrient availability, and delayed leaf senescence, which have made generalizations difficult (Welter 1989, Peterson and Higley 1993, Peterson 2001).

Although a continuum of responses has been observed, many studies indicate that removal of either partial or entire leaves by insect herbivores increases photosynthetic rates of remaining leaf tissue (Welter 1989). Reductions in photosynthetic rates have been

observed, but the reductions were temporary (Aldersfelder and Eagles 1976, Hall and Ferree 1976, Li and Proctor 1984). Reductions also were observed at the canopy level and were caused by decreased leaf-area indices, smaller leaf size, and decreased light interception (Detling et al. 1979, Boote et al. 1980, Ingram et al. 1981).

Several researchers also have observed no changes in photosynthetic rates of remaining leaf tissue of injured leaves in response to insect defoliation (Davidson and Milthorpe 1966; Poston et al. 1976; Syvertsen and McCoy 1985; Welter 1989, 1991; Higley 1992; Peterson et al. 1992, 1996; Peterson and Higley 1996, Burkness et al. 1999). These responses suggest that the photosynthetic apparatus of many plant species is not affected directly by leaf-mass consumption injury. The principal effect of this injury type seems to be the reduction of photosynthesizing leaf area, not reduction or enhancement of photosynthetic capacity of remaining tissue of injured leaves (Peterson and Higley 1996). (See Welter 1989 and Peterson 2001 for a review of physiological mechanisms underlying plant gas exchange responses to arthropod injury.)

In previous studies on the cultivated legume species soybean, *Glycine max* (L.) Merrill, and alfalfa, *Medicago sativa* L., researchers have observed that leaf-mass consumption by numerous insect species does not affect photosynthetic rates of remaining leaf tissue (Poston et al. 1976; Hammond and Pedigo 1981; Ingram et al. 1981; Ostlie and Pedigo 1984; Welter 1991; Higley 1992; Peterson et al. 1992; Peterson and Higley 1993, 1996; Peterson 2001). However, defoliation of whole plants did alter the pattern of normal progressive leaf senescence of soybean and alfalfa plants (Higley 1992, Peterson et al. 1992). Consequently, it is critical to characterize plant responses to insect injury to specific organizational levels, such as cells, leaves, whole plants, and plant canopies (Peterson 2001).

Pedigo et al. (1986) and Higley et al. (1993) emphasized the utility of categorizing biotic stressors based on injury type and plant response, rather than on biological classification. Peterson and Higley (2001) argued that a physiologically based approach can "provide a common language for characterizing plant stress and is essential for integrating understandings of stress." Furthermore, they argued that homogeneities of plant response to specific injury types (also known as injury guilds) can be used effectively to address basic questions, such as plant-defense evolution and speciation, and applied questions, such as crop yield-loss functions and economic injury levels (EILs).

Characterizing the physiological mechanisms underlying plant responses to arthropod injury is crucial to explain adequately yield loss and develop general models of response. Indeed, Welter (1989) stated, "... if researchers are not going to be fettered forever to repeating experiments for each plant–arthropod combination, then a more general understanding of plant responses is required."

Despite the research to date that has addressed plant gas exchange responses to insect herbivory, relatively few plant species have been examined. Currently, it is unknown whether responses to leaf-mass removal by insects vary among plant families and species (Peterson et al. 1996). This knowledge is crucial if the hypothesis of a generalized model of plant response is to be supported or refuted. Within the legume family, only alfalfa and soybean have been examined in sufficient detail. Therefore, the specific goal of this study was to test the hypothesis that gas exchange responses to leaf-mass consumption in a broad range of cultivated legumes do not differ from those of soybean and alfalfa. More generally, results from this experiment were used to evaluate whether there is a generalized plant gas exchange response to leaf-mass consumption injury.

Materials and Methods

Twelve experiments were conducted from May through July 2003 at the Montana State University Plant Growth Center Greenhouses, Bozeman, MT. Plants were seeded on 13 March 2003 in 20-cm-diameter plastic pots containing equal parts of silt loam soil,

washed concrete sand, and Canadian sphagnum peat moss. The greenhouse-bay temperature was maintained at 22°C. After emergence, plants were thinned to two per pot. Plants were watered four times weekly and fertilized once per week by using Peters General Purpose 20–20–20 at 200 ppm.

The species and cultivars used were alfalfa; *M. sativa* Cimarron; *Medicago scutellata* (L.) Sava; *Medicago truncatula* Gaertner Paraggio; yellow blossom sweetclover, *Melilotus officinalis* (L.) Pallas; alsike clover, *Trifolium hybridum* L.; and medium red clover, *Trifolium pratense* L. The species were chosen because they represent a variety of life history traits, ploidy levels, genera, and species within genera. Furthermore, they were used because seed were readily available and they are cultivated species, ensuring reliability in greenhouse propagation.

Alfalfa 'Cimarron' (PVP7900092) is a genetically broad-based, self-incompatible, cross-pollinated, semi-dormant, widely adapted perennial tetraploid. Cimarron has resistance to spotted alfalfa aphid, *Therioaphis trifolii* (Monell) f. maculata, and pea aphid, *Acyrtosiphon pisum* (Harris), and the diseases bacterial wilt, anthracnose, and phytophthora root rot (Miller and Melton 1983). *M. scutellata* 'Sava' is a self-pollinated, tetraploid annual, and has good tolerance to sitona weevil, *Sitona humeralis* Stephens; spotted alfalfa aphid; and the blue alfalfa aphid, *Acyrtosiphon kondoi* (Shinji) (SADAF 1980). *M. truncatula* 'Paraggio' is a self-pollinated, diploid annual, and has good tolerance to spotted alfalfa aphid and blue alfalfa aphid, but not to pea aphid (SADAF 1982). The alsike and red clover entries were from "common", variety-not-stated, seed lots. Nearly all alsike clover marketed in the United States is diploid, self-incompatible, and cross-pollinated. Alsike clovers typically are weakly perennial, but with resistance to northern and southern anthracnose (Townsend 1985). Red clover is more strongly perennial than is alsike clover. Red clover typically is diploid, although a few tetraploid cultivars have been developed (Smith et al. 1985). It is self-incompatible and cross-pollinated, and populations vary widely for resistance to northern and southern anthracnose. Yellow blossom sweetclover is a cross-pollinated diploid, typically with a biennial growth habit.

Each legume species was used in a separate experiment and each experiment was replicated twice. A randomized complete block, repeated measures design was used with five metal halide lamps (1000 W) with a photoperiod of 14:10 (L:D) h serving as the blocking factors. Two or three pots (depending on number of treatments), each serving as an experimental unit, were arranged under each lamp. Treatments were randomly assigned to the pots.

The first experiment for each legume species included two treatments: no injury (control) and clip injury (50% leaf tissue removed with scissors). The second experiment included three treatments: no injury (control), clip injury (50% leaf tissue removed scissors), and actual insect defoliation. The three-treatment experiment was not conducted for *M. truncatula* because the leaves and petioles were too small

Table 1. Mean photosynthetic rates ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$) \pm SEM by legume species, experiment, and time after cessation of injury

Species	Treatment	Exp. 1		Exp. 2	
		1 h	24 h	1 h	24 h
<i>M. sativa</i>	Control	34.6 \pm 1.29	17.8 \pm 0.54	26.5 \pm 0.94	17.7 \pm 2.55
	Clip ^a	31.7 \pm 1.85	17.5 \pm 2.77	23.3 \pm 2.34	18.4 \pm 0.34
	FAW (27.6 \pm 4.5) ^b			21.5 \pm 1.49	20.8 \pm 1.43
	<i>F</i>		1.08		1.36
	<i>P</i> > <i>F</i>		0.51		0.31
<i>M. scutellata</i>	Control	32.3 \pm 0.59	26.8 \pm 1.13	19.8 \pm 2.82	20.4 \pm 2.74
	Clip	31.4 \pm 1.63	29.4 \pm 1.11	20.0 \pm 2.99	20.9 \pm 3.02
	FAW (8.4 \pm 2.5)			18.3 \pm 1.62	19.7 \pm 2.54
	<i>F</i>		0.43		0.11
	<i>P</i> > <i>F</i>		0.55		0.9
<i>M. truncatula</i>	Control	32.4 \pm 1.50	28.8 \pm 0.85		
	Clip	33.4 \pm 0.71	29.9 \pm 0.74		
	FAW				
	<i>F</i>		1.05		
	<i>P</i> > <i>F</i>		0.36		
<i>T. hybridum</i>	Control	24.9 \pm 1.72	16.3 \pm 1.45	22.3 \pm 1.42	21.9 \pm 1.73
	Clip	25.9 \pm 2.44	14.8 \pm 2.27	21.2 \pm 1.22	21.0 \pm 1.76
	FAW (19.6 \pm 4.9)			19.9 \pm 2.18	22.5 \pm 2.03
	<i>F</i>		0.05		0.51
	<i>P</i> > <i>F</i>		0.83		0.62
<i>T. pratense</i>	Control	23.4 \pm 0.60	13.4 \pm 2.09	25.1 \pm 1.98	24.8 \pm 1.55
	Clip	27.0 \pm 1.78	14.2 \pm 1.59	20.6 \pm 2.37	23.3 \pm 1.02
	FAW (27.6 \pm 4.0)			23.6 \pm 1.00	23.9 \pm 0.98
	<i>F</i>		16.82		2.15
	<i>P</i> > <i>F</i>		0.02		0.18
<i>M. officinalis</i>	Control	27.5 \pm 3.13	18.9 \pm 2.01	24.9 \pm 1.64	19.2 \pm 2.97
	Clip	34.7 \pm 2.51	21.5 \pm 1.56	28.1 \pm 2.17	20.7 \pm 1.92
	FAW (18.0 \pm 7.5)			24.2 \pm 2.25	20.9 \pm 1.60
	<i>F</i>		2.86		0.96
	<i>P</i> > <i>F</i>		0.17		0.43
	<i>df</i>		1, 4		2, 8

^a Clip, 50% simulated insect defoliation of single leaf per plant.

^b FAW, fall armyworm defoliation. Numbers in parentheses are mean percentage defoliation per individual leaf \pm SEM.

for the insect defoliator (see below). All plants used were in the late vegetative or early reproductive stage.

For simulated and actual insect defoliation, injury was limited to a single leaf per plant, which was chosen at random from the upper one-third of the plant and marked. Simulated insect injury (clip injury) treatments were imposed by removing leaf tissue on each leaflet with scissors. The distal half of each leaflet was removed. For the actual insect defoliation treatment, third and fourth instars of fall armyworm, *Spodoptera frugiperda* (J.E. Smith), previously reared on artificial diet were placed on a leaf and allowed to feed. Larvae that moved off of a leaf were returned to the marked leaf or were replaced with new larvae. Larvae were starved for at least 12 h before being placed on a leaf. Consequently, defoliation proceeded quickly and larvae were removed from marked leaves from 1 to 4 h after being placed on the leaf. The percentage defoliation on the injured leaf was visually estimated (Table 1). The clip injury treatments were imposed as the larvae were being placed on the leaves.

For each marked leaf, gas exchange parameters (photosynthesis, stomatal conductance, intercellular CO₂, and transpiration) and leaf temperatures were measured using a portable photosynthesis system

(model LI-6400, 0.79-cm² Arabidopsis chamber, LI-COR, Lincoln, NE). Small leaf size and defoliation necessitated the use of the 0.79-cm² chamber. Measurements were recorded on a treated leaflet within 2 h of solar noon directly under the lamps; photosynthetically active radiation was >1,200 $\mu\text{mol photons}/\text{m}^2/\text{s}$ during all measurements and chamber CO₂ was 400 ppm, generated from a 12-g CO₂ cylinder connected to the LI-6400. For the simulated and actual insect defoliation treatments, an injured leaflet was measured. Measurements from each marked leaf were recorded 1 and 24 h after injury so that we could detect any transient effects of the defoliation.

Time-Series Responses. An additional experiment was conducted on *T. pratense* to characterize single leaf responses to defoliation over a longer period than 24 h and to determine whether defoliation impacts specific physiological aspects of the photosynthetic process under conditions of varying light and intercellular CO₂. A light curve (gas exchange variables measured at light intensities ranging from 0 to 2,000 $\mu\text{mol photons}/\text{m}^2/\text{s}$) and an A-Ci curve (gas exchange variables measured at CO₂ concentrations ranging from 50 to 600 ppm) were observed for each experimental unit. Only four replications were used for the

A-Ci curve determinations because of the considerable length of time needed to record those measurements. Two treatments (control and clip injury) were imposed following the protocol discussed previously, except that a 6-cm² chamber was used with the portable photosynthesis system. The 6-cm² leaf chamber was used because it could be fitted with a variable intensity light source, which is essential for measuring light curve responses. Gas exchange measurements were recorded 1, 7, and 19 d after injury. Light and A-Ci curve responses only were recorded 1 d after injury. *T. pratense* was used for this experiment because the leaves were sufficiently large to occupy the entire area of the chamber.

Because gas exchange variables were observed on the same leaf over time, statistical analyses were conducted using a repeated measures analysis of variance (ANOVA) ($\alpha = 0.05$). Light curve and A-Ci data were recorded 1 d after injury only; therefore, those data were analyzed using ANOVA ($\alpha = 0.05$).

Results and Discussion

Mean insect defoliation percentages (defoliation per individual leaf) over all plant species and experiments ranged from 8.4 to 27.6 (Table 1). Percentage of defoliation by fall armyworm larvae was low for *M. scutellata* most likely because that species is characterized by densely pubescent leaves and stems, which may have reduced palatability.

In all experiments, actual and simulated insect defoliation did not affect stomatal conductance, intercellular CO₂, transpiration rates, or leaf temperatures. In 10 of the 11 experiments, there were no significant photosynthetic differences among the three treatments. Actual and simulated insect defoliation did not significantly affect photosynthetic rates (Table 1). Photosynthetic rates for the simulated insect defoliation treatment were significantly greater than the undefoliated control at 1 h after injury for *T. pratense* in the first experiment. However, there were no significant differences at 24 h in that experiment or at either time in the second experiment. Furthermore, *T. pratense* photosynthetic rates were not significantly different between treatments at any time during the time-series experiment (see below).

Time-Series Responses. Defoliation did not significantly affect any of the *T. pratense* gas exchange parameters at 1, 7, or 19 d after injury (Table 2). Photosynthetic rates for both treatments were similar at 1 and 7 d after injury but were reduced at 19 d after injury, which likely reflected normal progressive leaf senescence.

Light-Response Curves. The relationship between photosynthesis and photosynthetic photon flux density (PPFD) is shown in Fig. 1. Photosynthesis for leaves from both treatments was maximized at a similar PPFD. The initial slope of the light-response curve indicates of quantum efficiency of CO₂ assimilation (maximum efficiency with which a leaf can use an absorbed photon for CO₂ assimilation) (Baker and Ort 1992). The initial slopes (0–100 $\mu\text{mol photons/m}^2/\text{s}$)

Table 2. Mean gas exchange responses \pm SEM of *T. pratense* to defoliation injury

Treatment	1 d	7 d	19 d
CO ₂ exchange rate ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)			
Control	20.4 \pm 1.71	18.2 \pm 2.68	15.3 \pm 3.50
Clip	21.8 \pm 0.76	19.7 \pm 2.00	11.0 \pm 3.04
<i>F</i>		0.02	
<i>P</i> > <i>F</i>		0.90	
<i>df</i>		1, 4	
Stomatal conductance ($\text{mol H}_2\text{O}/\text{m}^2/\text{s}$)			
Control	0.38 \pm 0.07	0.37 \pm 0.10	0.28 \pm 0.10
Clip	0.44 \pm 0.04	0.41 \pm 0.12	0.21 \pm 0.07
<i>F</i>		0.02	
<i>P</i> > <i>F</i>		0.90	
<i>df</i>		1, 4	
Transpiration rate ($\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$)			
Control	7.12 \pm 0.56	3.96 \pm 0.59	3.96 \pm 0.95
Clip	7.67 \pm 0.33	4.28 \pm 0.60	3.36 \pm 0.79
<i>F</i>		0.01	
<i>P</i> > <i>F</i>		0.91	
<i>df</i>		1, 4	
Intercellular CO ₂ (ppm)			
Control	260 \pm 0.38	268 \pm 12.97	248 \pm 17.79
Clip	270 \pm 5.82	266 \pm 15.25	276 \pm 10.26
<i>F</i>		1.57	
<i>P</i> > <i>F</i>		0.28	
<i>df</i>		1, 4	

for both treatments were not significantly different from each other ($F = 2.21$; *df* 1, 4; $P > F = 0.21$). The light compensation points (the intensity of light in which CO₂ assimilation is equal to respiration) for defoliated and undefoliated leaves were not significantly different ($F = 0.39$; *df* 1, 4; $P > F = 0.56$). The light-response curves revealed that leaf-mass consumption injury did not impact the photosynthetic electron transport chain.

A-Ci Response Curves. The relationship between photosynthesis and intercellular CO₂ is shown in Fig. 2. Photosynthesis for both leaves from both treatments was similar across all intercellular CO₂ concentrations. The initial slope of the A-Ci curve provides a measure

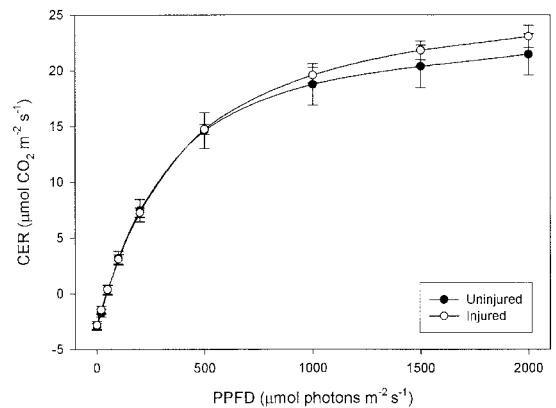


Fig. 1. Relationship between photosynthetic rates (as carbon exchange rate, CER) and PPFD for injured and uninjured *T. pratense* leaves. Vertical, capped lines indicate \pm SEM.

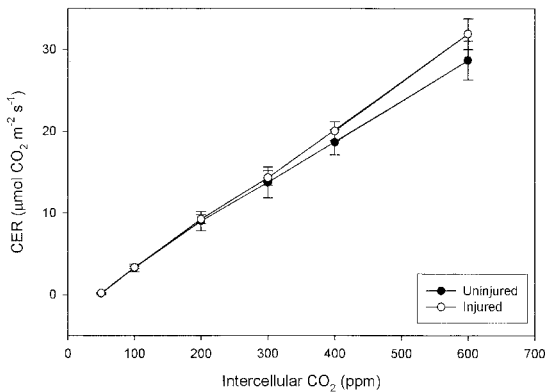


Fig. 2. Relationship between photosynthetic rates (as carbon exchange rate, CER) and intercellular CO₂ for injured and uninjured *T. pratense* leaves. Vertical, capped lines indicate \pm SEM.

of the dark reaction (Calvin cycle) function. In particular, it indicates Rubisco efficiency and ribulose-bisphosphate (RuBP) regeneration (Sharkey 1985). The initial slopes (50–200 ppm) for both treatments were not significantly different from each other ($F = 0.05$; df 1, 3; $P > F = 0.84$). The A-Ci curve responses revealed that there was no significant impact of defoliation on Rubisco activity or RuBP regeneration.

Peterson and Higley (1996) noted subtle, transient changes in gas exchange parameters in soybean leaves within a few hours after defoliation injury. These perturbations most likely were because of water loss from injured tissue and did not cause significant reductions in photosynthetic or stomatal conductance rates. In our study, we did not observe any significant transient changes in either H₂O or CO₂ exchange.

Despite a variety of life history traits and ploidy levels, defoliation of single leaves on the six species resulted in similar responses. Plant gas exchange variables were not significantly affected by either simulated or actual insect defoliation. In this study, the primary effect of single-leaf defoliation on photosynthesis was to reduce the amount of photosynthetic leaf area rather than reduce or increase photosynthetic rates of remaining tissue on a defoliated leaf. This result is similar to previous findings on a number of varieties of soybean and alfalfa (Poston et al. 1976; Hammond and Pedigo 1981; Ingram et al. 1981; Ostlie and Pedigo 1984; Welter 1991; Higley 1992; Peterson et al. 1992; Peterson and Higley 1993, 1996; Peterson 2001). Consequently, this study supports the hypothesis that there is a common modality of response by legumes to the insect leaf-mass consumption injury guild.

The issue of injury guilds and the results from this study potentially are important for pest management applications. One of the key requirements for developing multiple-species EILs is that the pest species must produce a similar type of injury (Higley et al. 1993). Based on results from this study and previous studies, homogeneities in photosynthetic responses

occur between different leaf-mass consumers and different cultivated legume species. This homogeneity can then be used to create physiologically based injury guilds for multiple pests and crop species.

Because there were no significant differences between actual and simulated injury, it is possible to simulate insect leaf-mass consumption for these legume species. This is important because simulating insect defoliation often allows for a more accurate quantification of injury than other methods, including using actual insects. However, if the objective of a study is to characterize whole plant and yield or fitness responses to leaf-mass consumption, simulation techniques must adequately reproduce the spatial and temporal pattern of injury on the plant (Ostlie 1984, Pedigo et al. 1986).

The common modality of physiological response to the leaf-mass consumption injury guild most likely is not limited to legumes. Higley et al. (1993), Peterson and Higley (1993), and Peterson and Higley (2001) argued that homogeneities of physiological response to distinct injury types most likely are common. Peterson et al. (1996) and Burkness et al. (1999) did not observe photosynthetic rate differences after insect leaf-mass consumption on apple, *Pyrus malus* L., and crabapple, *Pyrus coronaria* L., and cucumber, *Cucumis sativus* L., respectively. Welter (1991) observed no differences in photosynthetic rates after tomato hornworm, *Manduca quinque maculata* (Howarth) defoliation on tomato, *Lycopersicon lycopersicum* (L.).

Even though homogeneities of physiological response to defoliation injury were observed in this study and previous studies, there are known exceptions. On soybean and dry bean, *Phaseolus vulgaris* L., Peterson et al. (1998) observed photosynthetic rate reductions in remaining leaf tissue after defoliation by Mexican bean beetle, *Epilachna varivestis* Mulsant, adults and larvae. Both adult and larval defoliation results in skeletonization of the leaf, which is physically different than defoliation by other species. Because gas exchange responses to Mexican bean beetle were different than other defoliators, Peterson et al. (1998) concluded that Mexican bean beetle injury could not be placed in the leaf-mass consumption injury guild.

Delaney (2003) observed photosynthetic rate reductions in remaining milkweed, *Asclepias syriaca* L., leaf tissue after defoliation by milkweed tussock moth, *Euchaetes egle* (Drury), and monarch butterfly, *Danaus plexippus* (L.), larvae. Zangerl et al. (2002) observed photosynthetic rate reductions in remaining wild parsnip, *Pastinaca sativa* L., leaves after defoliation by cabbage looper, *Trichoplusia ni* (Hübner). In these cases, there seems to be a tradeoff between active plant defense and the ability for individual leaves to be unaffected by defoliation. This hypothesis is further supported with assimilate sapping injury [Russian wheat aphid, *Diuraphis noxia* (Mordvilko)] and wheat, *Triticum aestivum* L. (Haile et al. 1999).

In this study, our results support the hypothesis that there is a generalized plant gas exchange response to leaf-mass consumption injury among the cultivated

legumes. Additionally, our results and previous studies suggest that this generalized plant response may extend to many species.

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