

Mexican Bean Beetle (Coleoptera: Coccinellidae) Injury Affects Photosynthesis of *Glycine max* and *Phaseolus vulgaris*

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ABSTRACT Based on previous photosynthesis studies, adult Mexican bean beetles, *Epilachna varivestis* Mulsant, produce a different physiological response to injury in soybean than other insect defoliators. In 1993 and 1994, we conducted experiments to determine the nature and extent of photosynthetic rate reductions in soybean, *Glycine max* (L.) Merrill, and dry bean, *Phaseolus vulgaris* L. We used a randomized complete block design for all experiments. In most experiments, treatments were an uncaged, uninjured leaflet; a caged, uninjured leaflet; and a caged, injured leaflet. Treatments were replicated >5 times. Experimental units were individual trifoliolate leaflets. Four to 8 larvae or adults were placed in each leaflet cage and allowed to feed for 6-18 h. After feeding, the insects and leaf cages were removed and gas exchange properties were determined. Both adults and larvae reduced photosynthetic rates of the remaining tissue of the injured leaflet on both soybean and dry bean. A significant linear relationship between photosynthetic rate and percentage injury was observed for both adult and larval injury. Injury reduced photosynthetic rates in all 6 soybean and dry bean cultivars used in the experiments. There was no recovery of photosynthetic rates after injury of an individual leaflet. Stomatal conductance rates were not consistently different between injured and uninjured leaflets. Intercellular CO₂ concentrations were similar or higher in injured leaflets. Consequently, reductions in photosynthesis do not seem to be attributable to stomatal limitations. Quantum efficiency was not affected by injury, indicating that light-harvesting structures were not perturbed. Therefore, our results suggest that the limitations to photosynthesis are attributable to the utilization of CO₂ or the supply or utilization of phosphate. Our findings suggest that the limitation is associated with RuBPcase, RuBP regeneration, or phosphate utilization.

KEY WORDS *Epilachna varivestis*, herbivory, defoliation, gas exchange, soybean, dry bean

RESEARCH ON BIOTIC stress and its effect on plant physiology is still in its infancy. Few researchers have attempted to synthesize responses of plants to biotic stresses (Welter 1989, Higley et al. 1993, Peterson and Higley 1993). The paucity of research on physiological responses to biotic stress is especially apparent in the area of arthropod-induced plant stress, even though insect and mite injury represents one of the most important types of biotic stress (Higley et al. 1993).

Injury by arthropods potentially can affect population dynamics and life history strategies of both herbivores and plants. Characterizing the influence of arthropods on plant gas exchange processes, such as photosynthesis, water vapor transfer, and respiration is important because these processes are crucial determinants of plant growth, yield, and fitness. Therefore, characterizing physiological responses to herbivores can "provide a common basis for understanding how plants respond to insect-induced stress" (Peterson and Higley 1993).

During field research in 1989 and 1990, individual leaflets of soybean, *Glycine max* (L.) Merrill, were exposed to injury by several insect herbivores, includ-

ing redlegged grasshoppers, *Melanoplus femurrubrum* (DeGeer); soybean loopers, *Pseudoplusia includens* (Walker); velvetbean caterpillars, *Anticarsia gemmatalis* (Hübner); green cloverworms, *Plathypena scabra* (F.); soybean leafminer adults, *Odontota horni* Smith; bean leaf beetle adults, *Ceratoma trifurcata* (Forster); and Mexican bean beetle adults, *Epilachna varivestis* Mulsant (L.G.H., unpublished data). Photosynthetic rates of the remaining leaflet tissue were not affected by herbivore injury, except for the injury by Mexican bean beetles. Mexican bean beetle injury significantly reduced photosynthetic rates of the remaining leaflets. However, the research discussed above and previous studies on soybean and several other plant species have demonstrated that both simulated and actual insect defoliation do not perturb photosynthetic rates of remaining tissue of individual, injured leaves (Welter 1989, 1991; Higley 1992; Peterson et al. 1992; Peterson and Higley 1993).

Injury by adult and larval Mexican bean beetles is physically different from injury by other lepidopteran and coleopteran soybean defoliators. Adults and larvae scrape, crush, and then consume leaf tissue, leaving both large and small leaf veins unconsumed, but often injured. Visually, the injured leaflet is "laced" or "skeletonized" (Edwards et al. 1994). Based on pre-

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liminary photosynthesis studies, it was evident that adult Mexican bean beetles also produce a different physiological response to injury in soybean than do other insect defoliators.

Substantial progress has been made in determining the physiological mechanisms responsible for reductions in photosynthetic activity. Sharkey (1985) identified 3 categories for all limitations to photosynthesis—the supply or utilization of CO₂, the supply or utilization of light, and the supply or utilization of phosphate. It is now possible to determine the physiological and biochemical limitations of photosynthesis to environmental conditions with both *in vitro* and *in vivo* techniques. Using ecophysiological instrumentation and biochemically based models (Farquhar and von Caemmerer 1982, Farquhar and Sharkey 1982, Sharkey 1985), researchers have determined the role of stomatal and nonstomatal limitations to photosynthesis in several plant systems (e.g., Sharkey and Seemann 1989, Bowden et al. 1990, Pennypacker et al. 1990, Ni and Pallardy 1992, Kicheva et al. 1994, Koch et al. 1994).

Specific biochemical limitations, such as ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBPCase) activity, ribulose 1, 5-bisphosphate (RuBP) regeneration, and triose phosphate utilization can be determined for C₃ species using a combination of assimilation-intercellular CO₂ response curves, quantum efficiency determinations, fluorescence measurements, and metabolite assays (Sharkey 1985). Only the metabolite assay is a destructive technique. Single photosynthesis measurements provide limited information concerning biochemical limitations to photosynthesis. Therefore, assimilation-CO₂ response curves and quantum efficiency determinations require multiple photosynthesis measurements in relation to changing CO₂ concentrations and light intensities, respectively (Sharkey 1985). Unfortunately, much of the work on photosynthetic responses to insect injury is based on single measurements, and studies describing gas exchange mechanisms are lacking (Peterson and Higley 1993).

Many studies have characterized photosynthetic limitations during drought stress. However, a few researchers have examined photosynthetic limitations to plant pathogens. Bowden et al. (1990) observed that photosynthesis rates of leaves of potato, *Solanum tuberosum*, were reduced by *Verticillium dahliae* Kleb., a vascular fungal pathogen. Using gas exchange measurements, light response curves, and CO₂ response curves, they concluded that photosynthetic reductions primarily were caused by stomatal closure. Conversely, Pennypacker et al. (1990) observed that photosynthetic reductions of alfalfa, *Medicago sativa* L., infected with *Verticillium albo-atrum* Reinke & Berth. were caused by a reduction in the total activity and amount of RuBPCase, and not by stomatal closure.

The initial research we conducted prompted several questions about the nature and extent of photosynthetic rate reductions after injury by the Mexican bean beetle. These questions are important to answer if we are to develop general models of plant response

to arthropod injury types. We addressed the following 7 questions: (1) Is larval injury similar to adult injury in its effect on photosynthesis? (2) Does injury reduce photosynthesis in another host species, dry bean, *Phaseolus vulgaris* L.? (3) Does injury reduce photosynthesis in different soybean and dry bean cultivars? (4) Does injury reduce photosynthesis at different stages of soybean and dry bean development? (5) Do injured leaflets compensate for injury over time? (6) Is the entire leaflet affected by injury? and (7) What are the physiological and biochemical mechanisms responsible for the reductions in photosynthesis?

Materials and Methods

Indeterminate soybean was planted in 1993 ('Kenwood') and 1994 ('Clark 3W' and 'Clark 5N') at field sites on the East Campus of the University of Nebraska, Lincoln. The soil is a Zook silty clay loam (fine, montmorillonitic, mesic Cumulic Haploaquoll). All sites were disked before planting. Row orientation was north-south. Row width was 76 cm and planting density was 25 plants per row-meter. (Table 1 contains specific information on cultivars, treatment replications, and blocking factors.)

For the greenhouse experiments, soybean (Kenwood and 'Resnick') and dry bean ('Beryl' and 'Flint') were planted in plastic pots (16 cm diameter) containing 2/3 silt loam soil and 1/3 sand. Each plant was grown individually in a pot under a high-pressure sodium lamp (400 W), with a photoperiod of 14:10 (L:D) h.

We used 9 experiments in both field and greenhouse environments to address our research questions (Table 1). We used a randomized complete block design for all experiments. In most experiments, treatments were an uncaged, uninjured leaflet; a caged, uninjured leaflet; and a caged, injured leaflet. Treatments were replicated at least 5 times. Experimental units were individual trifoliolate leaflets. Because photosynthetic rates vary considerably among leaves, all 3 leaflets per leaf typically were used as experimental units to reduce natural variability. Previous research (comparisons of caged leaflets with uncaged leaflets within a leaf, or with uncaged leaves of different plants) indicates that injury to 1 leaflet by insect defoliators, including Mexican bean beetles, does not affect photosynthetic rates of an adjacent leaflet (L.G.H., unpublished data).

In all experiments, young, fully expanded leaflets were enclosed in bridal veil fabric cages which intercepted <5% of photosynthetically active radiation (determined with a quantum sensor, Model 191SA, LI-COR, Lincoln, NE). Depending on the experiment, 4–8 larvae or adults were placed in each leaflet cage and allowed to feed for 6–18 h. After feeding, the insects and leaf cages were removed and gas exchange measurements were recorded.

Gas Exchange. Measurements were taken in full sunlight within 2 h of solar noon, or under high-pressure sodium greenhouse lamps. All measurements were taken at >1,400 μmol photons m⁻² s⁻¹. Gas

Table 1. Factors specific to each experiment (1993–1994)

Host	Year	Location	Stressor	Cultivar ^a	Stage ^b	Replications	Blocking factor	% Injury (\pm SE)
Soybean	1993	Field	Larvae	Kenwood	R4	6	Row	17.8 (4.25)
		Field	Adults	Kenwood	R5	6	Row	20.5 (3.24)
	1994	Greenhouse	Adults	Kenwood	R5	8	Light	17.9 (6.02)
		Greenhouse	Larvae	Resnick	R2	5	Plant	22.5 (5.28)
		Field	Adults	Clark 5N	R3	7	Plant	22.1 (4.31)
		Field	Adults	Clark 3W	R2	8	Plant	39.1 (6.62)
Dry bean	Greenhouse	Adults	Flint	R4	8	Plant	19.6 (3.89)	
	Greenhouse	Larvae	Flint	R3	8	Plant	26.2 (5.78)	
	Greenhouse	Larvae	Beryl	R3	8	Plant	21.9 (4.54)	

Each row represents an individual experiment.

^a Clark 3W and Clark 5N are indeterminate isolines. Clark 5N has 5 narrow leaflets per leaf; Clark 3W has 3 wide (typical) leaflets per leaf.

^b Developmental stages of soybean were determined according to the classification scheme of Fehr and Caviness (1977); stages of dry bean were determined according to the classification scheme of Nuland et al. (1983).

exchange measurements (net CO₂ exchange rate, stomatal conductance rate, transpiration rate, intercellular CO₂ concentration) were recorded using a portable photosynthesis system (Model LI-6200, LI-COR). We used either a 4-liter chamber enclosing the entire leaflet or a 1-liter chamber with restriction devices to cover and measure only the visually uninjured portion of an injured leaflet. The leaf area measured with the use of restrictors was 7.6 cm².

Stomatal conductance and transpiration rates were recorded using a steady-state porometer (Model LI-1600, LI-COR). The steady-state porometer provides a more accurate estimate of stomatal conductance than the portable photosynthesis system because the photosynthesis system will incorporate water loss from the cut leaf tissue into an estimate of transpiration, stomatal conductance, and boundary layer conductance. The steady-state porometer estimates only stomatal conductances and boundary layer conductances of the remaining tissue because the chamber is small enough to enclose only remaining, uninjured tissue.

Respiration measurements were recorded by covering the leaf chamber with a photographic film changing bag, which intercepted all light. Before measurements were recorded, the leaflet was allowed to equilibrate in the dark until CO₂ production was positive (\approx 20 s). The changing bag did not cause leaflet temperature to increase during remaining, uninjured tissue. Respiration measurements were recorded on Kenwood soybean in 1993 only.

In 1994, we used a solid-state lighting intensity system (Model QBeam 2001-A, Quantum Devices, Barneveld, WI) to adjust the photosynthetic photon flux density incident on each leaflet before photosynthetic parameters were measured. Photosynthetic responses to varying photosynthetic photon flux density can be used to characterize several parameters associated with photochemical efficiency (Baker and Ort 1992). Leaflets were placed in a 1-liter leaf chamber with area restrictors for measurements and allowed to equilibrate to the specific light intensity for \approx 1 min. Gas exchange parameters were measured at 50, 100, 400, 800, 1,200, 1,400, and 1,700 μ mol photons m⁻² s⁻¹. After each measurement at a specific photosynthetic

photon flux density, the chamber was opened so that the CO₂ inside the chamber could return to ambient conditions. Light response curves were determined only for Clark 3W soybean.

After final gas exchange measurements were recorded, the leaflets were removed and area was quantified using an image analysis system consisting of a color video camera (3CCD, Model DXC-960MD, Sony), an image digitizing board (Targa+, Truevision, Indianapolis, IN) and imaging analysis software (Mocha, Jandel Scientific, San Rafael, CA). Using this system, the percentage injury (skeletonization) could be determined. Therefore, photosynthesis values reflect rates of only the remaining, living tissue of an injured leaflet. Leaf areas of injured leaflets can not be determined with conventional leaf area meters because the skeletonization injury produces small holes that cannot be recognized by the sensors of area meters.

Statistical Analyses. Statistical analyses were conducted using analysis of variance (ANOVA), with treatment means separated by protected least significant difference (LSD) ($P = 0.05$). Linear and non-linear regression analyses were conducted to determine the relationship between injury and photosynthetic rate.

Results and Discussion

Injury from Mexican bean beetle adults and larvae ranged from 0.01 to 70% reduction in leaf area over all experiments. In all experiments except 1, photosynthetic rates of caged, uninjured leaflets were not significantly different from uncaged, uninjured leaflets. Therefore, the mesh cages did not affect photosynthetic rates. Additionally, caged leaflets and leaves have not affected gas exchange rates in previous studies on several plant species, including soybean, sunflower; *Helianthus annuus* L.; apple, *Pyrus malus* L.; crabapple, *Pyrus coronaria* L.; common milkweed, *Asclepias syriaca* L.; and tickclover, *Desmodium illinoense* A. Gray. (L.G.H., unpublished data; Peterson et al., 1996).

Adult and Larval Injury and Photosynthesis. Both adults and larvae reduced photosynthetic rates of the injured leaflet on soybean and dry bean. A significant

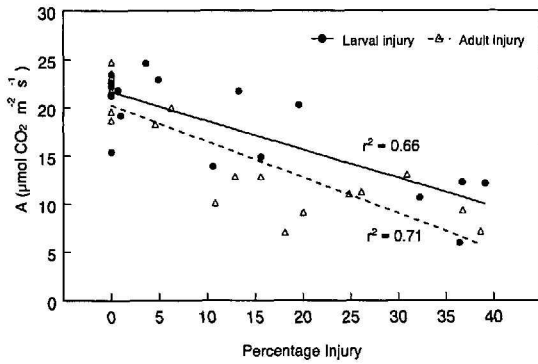


Fig. 1. The relationship between assimilation and percentage of Mexican bean beetle injury per leaflet. Data are from Kenwood soybean, 1993.

linear relationship between photosynthetic rate and percentage injury was observed for both adult and larval injury (Fig. 1). Higher-order polynomial relationships between photosynthetic rate and percentage injury were not significant. Increasing injury decreased photosynthetic rates linearly in 6 of the 9 experiments (Table 2). Therefore, not only do larvae and adults produce injuries that are visually similar, but they also produce injuries that are physiologically similar.

Injury and *P. vulgaris*. Photosynthetic responses of dry bean to Mexican bean beetle injury were similar to soybean (Fig. 2). Injury by larvae and adults significantly reduced photosynthesis compared with uninjured leaflets in all 3 experiments. Fundamental differences in leaf morphology (such as trichome density) between soybean and dry bean did not seem to affect the responses of individual leaflets to injury.

Injury and Plant Cultivars. Mexican bean beetle injury reduced photosynthetic rates in all soybean and dry bean cultivars used in these experiments (Fig. 2). Although we used only a few cultivars, it seems likely that many cultivars would exhibit reductions in photosynthesis to this type of injury, given that similar responses were observed between the 2 species. However, different cultivars may be able to compensate for injury better than others.

Injury and Plant Developmental Stages. Injury reduced photosynthetic rates of soybean in the R2, R3, R4, and R5 growth stages (Table 1). Further, photo-

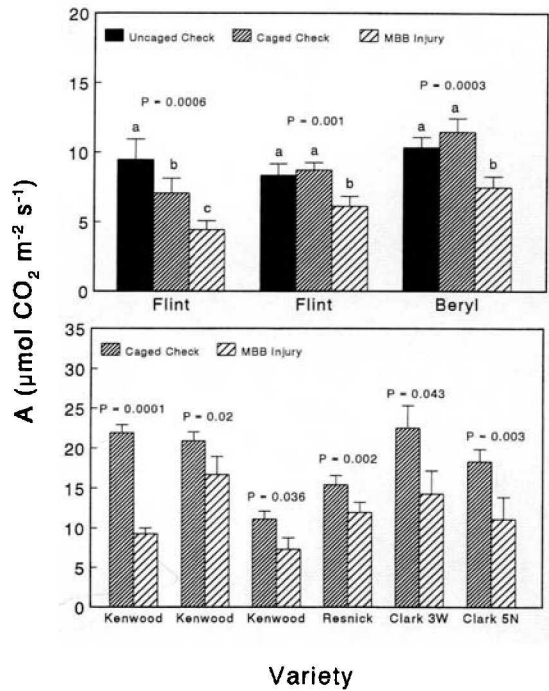


Fig. 2. Photosynthetic responses of dry bean (top graph) and soybean (bottom graph) cultivars to Mexican bean beetle injury. MBB, Mexican bean beetle. Vertical, capped lines are standard error. Means followed by the same letter are not significantly different ($P = 0.05$) according to protected LSD test. Data are from 1994.

synthetic rate reductions also occurred in dry bean in the R3 and R4 growth stages (Table 1). Although vegetative stages were not studied, we believe that these stages would respond similarly given that individual leaflets do not have unique anatomies at different stages of development.

Injury and Temporal Responses. In 2 experiments (1 field and 1 greenhouse experiment), we measured photosynthetic responses of soybean to injury over time after the removal of beetles. Results from both experiments indicate that there was no recovery of photosynthetic rates after injury of an individual leaflet (Fig. 3). In the greenhouse experiment, photosynthetic rates of injured leaflets were significantly lower than uninjured leaflets up to 31 d after injury ceased.

Table 2. Linear relationships between percentage injury per leaflet and photosynthetic rate for each experiment

Host	Year	Location	Stressor	Cultivar	Slope	r^2	$P > F$
Soybean	1993	Field	Larvae	Kenwood	-0.2972	0.66	0.0001
		Field	Adults	Kenwood	-0.3772	0.71	0.0001
	1994	Greenhouse	Adults	Kenwood	-0.2079	0.78	0.0001
		Field	Adults	Clark 5N	-0.2084	0.48	0.003
		Field	Adults	Clark 3W	-0.2978	0.24	0.075
Dry bean	Greenhouse	Larvae	Resnick			NS	
	Greenhouse	Larvae	Flint	-0.0812	0.46	0.005	
	Greenhouse	Adults	Flint			NS	
	Greenhouse	Larvae	Beryl			NS	

Each row represents an individual experiment. NS, not significant.

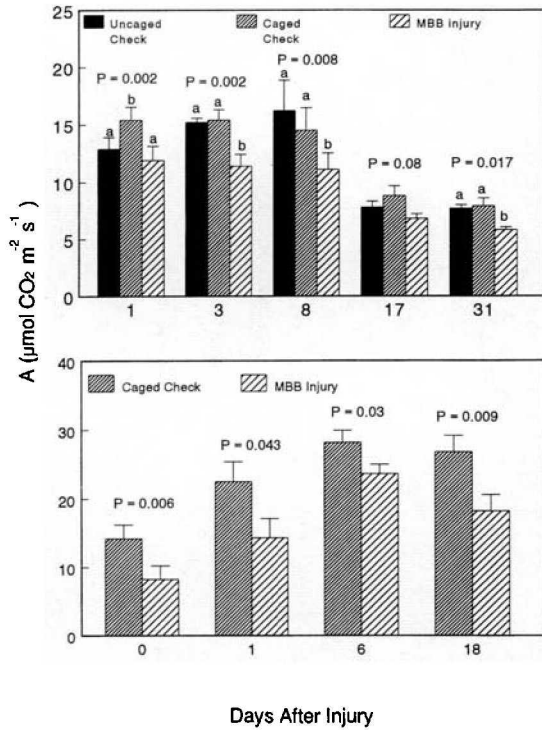


Fig. 3. Photosynthetic responses of soybean leaflets to Mexican bean beetle injury over time after the cessation of injury. MBB, Mexican bean beetle. Vertical, capped lines are SE. Means followed by the same letter are not significantly different ($P = 0.05$) according to protected LSD test. Data are from 1994.

In the field experiment, photosynthetic rates of injured leaflets were significantly lower than uninjured leaflets up to 18 d after injury ceased. In both experiments, there did not seem to be recovery of photosynthetic rates even from leaflets that experienced only 5–10% injury (Fig. 3). These data suggest that injury reduces the gas exchange capacity of the leaflet until it undergoes normal, progressive photosynthetic and physical senescence.

Injury and Intralesion Responses. By using a 1-liter chamber with restrictors to cover only an uninjured portion of an injured leaflet, we were able to determine if the entire leaflet was affected by the injury or if only the areas immediately surrounding the injury, or isolated by the injury, were affected. Typically, the areas measured were at the basal portion of the leaflet adjacent to the midrib vein. Using both a 1-liter chamber with restrictors and a 4-liter chamber, we observed significant reductions in photosynthetic rates between uninjured leaflets and injured leaflets (Fig. 4). Additionally, we compared the difference in percentage reduction of photosynthesis between controls and injured leaflets for the 4-liter method and the 1-liter method. Percentage reduction of photosynthesis between the 2 methods was not significantly different ($F = 0.01$; $df = 1, 6$; $P > F = 0.98$). These findings suggest

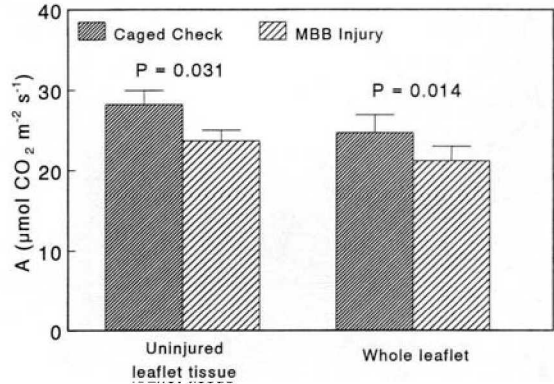


Fig. 4. Photosynthetic responses of soybean leaflets to Mexican bean beetle injury. 4-liter chamber enclosed the entire injured leaflet. 1-liter chamber method used restrictors to isolate and measure gas exchange of 7.6 cm² of visually uninjured tissue on an injured leaflet. MBB, Mexican bean beetle. Vertical, capped lines are standard error. Data are from 1994.

that the effects of injury to 1 portion of the leaflet are manifested across the entire leaflet.

Typically, areas of leaflets that are injured by both adults and larvae dry completely in 1 or 2 d. The dried, lacy tissue drops from the leaflet and the leaflet continues to function until senescence. Because we observed no recovery after injury (and after the loss of dead tissue), and because we observed photosynthesis reductions in visually uninjured tissue, our results suggest that the injury affects the entire leaflet.

Our findings for individual leaflets most likely can not be extrapolated to whole plants or plant populations because previous studies indicate that plants respond differently to arthropod injury at different levels of biological organization (Peterson and Higley 1993). For example, photosynthetic rates of individual alfalfa leaves injured by alfalfa weevil larvae are not significantly different from uninjured leaves (Peterson et al. 1992). However, when defoliation occurs across many leaves of a plant, the whole plant responds to defoliation by altering the photosynthetic senescence pattern of the remaining leaves. Therefore, photosynthetic rates are not altered by defoliation at the individual leaf level, but they are altered at the whole-plant level (Peterson et al. 1992). The delay in photosynthetic and physical leaf senescence seems to be a compensatory response to defoliation. These leaf and whole-plant responses also have been observed after leaf-mass consumption of soybean (Higley 1992).

Respiration. Because photosynthetic rates determined from the portable photosynthesis system are actually net carbon exchange rates, it could be argued that increases in respiration rates after injury are producing overall reductions in photosynthetic rates. However, respiration rates of the remaining tissue on injured leaflets were not significantly different ($F = 0.84$; $df = 1, 6$; $P > F = 0.31$) from uninjured leaflets. Indeed, although not significant, respiration rates of injured leaflets generally were lower than uninjured

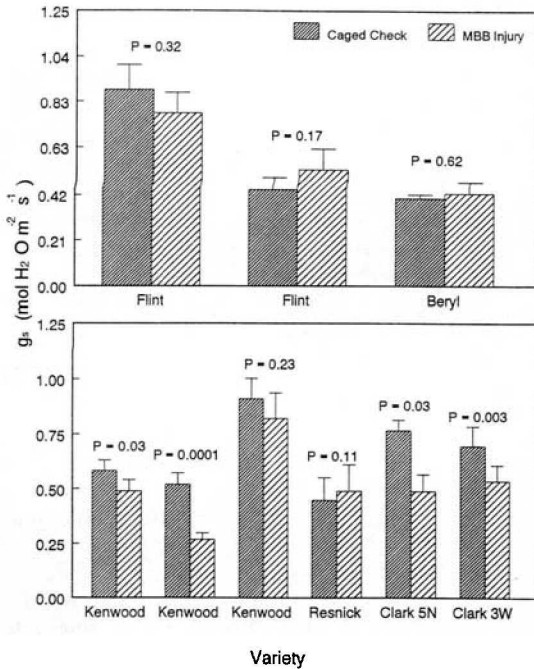


Fig. 5. Stomatal conductance of dry bean (top graph) and soybean (bottom graph) cultivars to Mexican bean beetle injury. MBB, Mexican bean beetle. Vertical, capped lines are SE. Data are from 1994.

leaflets. Consequently, respiration was unchanged or reduced most likely because of reductions in leaflet photosynthetic activity associated with injury.

Stomatal Conductance. Stomatal conductance rates were significantly different among treatments in only four of the nine experiments (Fig. 5). Where significant differences occurred among treatments, a linear relationship between percentage injury and stomatal conductance was observed in 3 of the 4 experiments (Table 3). As injury increased, stomatal conductance decreased linearly. However, the coefficient of determination, r^2 , was generally much less than the coefficient of determination for injury-photosynthetic rate regressions.

Intercellular CO_2 . Intercellular CO_2 concentrations in injured leaflets were not significantly different or were significantly greater than uninjured leaflets. Intercellular CO_2 concentrations typically were

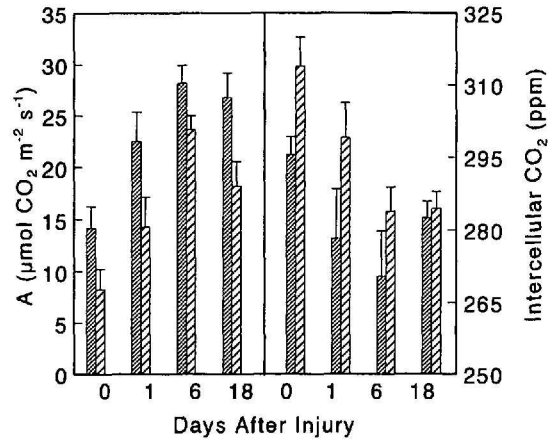


Fig. 6. Soybean (Clark 3W) assimilation (left y-axis) and intercellular CO_2 (right y-axis) over time after Mexican bean beetle injury. Bars with narrow lines represent caged control; bars with wide lines represent Mexican bean beetle injury. Vertical, capped lines are standard error. Data are from 1994.

greater in injured leaflets over time after injury (Fig. 6) and across all PPFs (Fig. 7). Therefore, CO_2 availability in the leaf mesophyll did not seem to be reduced by injury.

Light Response Curves. The relationship between photosynthesis and photosynthetic photon flux density is shown in Fig. 8. Injured leaflets had greater light compensation points (the photosynthetic photon flux density where the photosynthetic rate becomes positive) than uninjured leaflets, both immediately and 24 h after the cessation of injury. Although injured leaflets had lower photosynthetic rates than uninjured leaflets, photosynthesis of both treatments was maximized at a similar photosynthetic photon flux density. The initial slope of the light-response curve (Fig. 8) gives an indication of quantum efficiency of CO_2 assimilation (maximum efficiency with which a leaf can utilize an absorbed photon for CO_2 assimilation) (Baker and Ort 1992). The initial slopes ($50\text{--}400 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) for both treatments were not significantly different from each other (immediately after injury: $F = 0.02$; $df = 1, 6$; $P > F = 0.9$ 24 h after injury: $F = 0.87$; $df = 1, 5$; $P > F = 0.39$). Therefore, quantum efficiency was not reduced because of injury. Consequently, the limitation on photosynthesis

Table 3. Linear relationships between percentage injury per leaflet and stomatal conductance for each experiment

Host	Year	Location	Stressor	Cultivar	Slope	r^2	$P > F$	
Soybean	1993	Field	Larvae	Kenwood	-5.6	0.32	0.003	
		Field	Adults	Kenwood	-7.35	0.32	0.015	
	1994	Greenhouse	Adults	Kenwood				NS
		Field	Adults	Clark 5N	-6.39	0.4	0.011	
		Field	Adults	Clark 3W				NS
		Greenhouse	Larvae	Resnick				NS
Dry bean	Greenhouse	Larvae	Flint				NS	
		Adults	Flint				NS	
	Greenhouse	Adults	Flint				NS	
		Larvae	Beryl				NS	

Each row represents an individual experiment. NS, not significant.

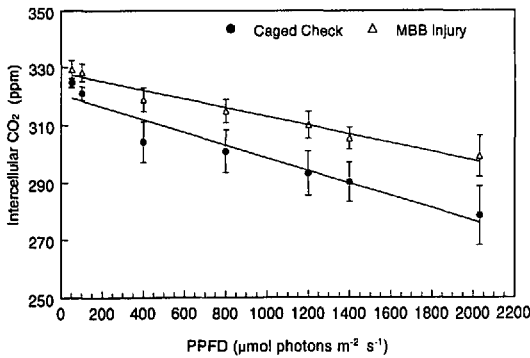


Fig. 7. The relationship between intercellular CO₂ and photosynthetic photon flux density (PPFD) of Mexican bean beetle injured and uninjured soybean leaflets (Clark 3W). MBB, Mexican bean beetle. Vertical, capped lines are standard error. Data are from 1994.

most likely was not because of damage to the photosynthetic electron transport chain.

Mechanisms Underlying Photosynthetic Rate Reductions. Our findings provide insights into the nature

of photosynthetic reductions after injury by Mexican bean beetle adults and larvae. Stomatal conductance rates were not consistently different between injured and uninjured leaflets. Additionally, intercellular CO₂ concentrations were similar or higher in injured leaflets. Therefore, the CO₂ supply into the leaf was not limited by stomatal closure. Consequently, stomatal limitations do not seem to be responsible for reductions in photosynthesis. Rather, the reductions in stomatal conductance of injured leaflets, when they occurred, seemed to be attributable to the reductions in photosynthesis. Therefore, stomatal conductance rates were tracking or following photosynthetic rates.

If stomatal effects are not responsible for the limitations to photosynthesis in soybean and dry bean, nonstomatal effects must be responsible. The nonstomatal, or mesophyll limitations, can be characterized further as light reaction limitations (the supply or utilization of light) or dark reaction limitations (the utilization of CO₂ or the supply or utilization of phosphate). The light reactions of photosynthesis do not seem to be affected by the injury. Quantum efficiency was not affected by injury, indicating that light-harvesting structures, as part of the Hill reaction, were not perturbed. Therefore, adenosine triphosphate and nicotinamide adenine dinucleotide generated as part of the light reaction were not limiting photosynthesis (as indicated by light-response curves).

By eliminating the possibility of reduced CO₂ availability and light-reaction limitations, we know that the limitations to photosynthesis must be attributable to the utilization of CO₂ or the supply or utilization of phosphate. Therefore, the limitation may be associated with RuBPCase activity, RuBP regeneration, or phosphate utilization. RuBP regeneration can be affected by the photosynthetic electron transport chain which produces adenosine triphosphate and nicotinamide adenine dinucleotide, or by insufficient capacity of the carbon reactions of the photosynthetic carbon reduction cycle (Sharkey 1985). Because our results suggest that photosynthetic electron transport is not limiting, RuBP seems to be limited by alterations in metabolite pools associated with the photosynthetic carbon reduction cycle. Several carbon compounds involved in the carbon reduction cycle include fructose 1,6-bisphosphate, triose phosphate, fructose 6-phosphate, ribose 5-phosphate, ribulose 5-phosphate, ribulose 1,5 bisphosphate, and 3-phosphoglycerate. Regulation of these metabolites include enzymes, allosteric regulation, and pH (Sharkey 1985). However, the precise mechanisms of regulation are still unclear.

Future research needs to include quantitative assays for RuBPCase, RuBP, as well as other carbon reduction cycle metabolites to verify that >1 metabolites or the enzyme is limiting photosynthesis. Additionally, fluorescence parameters should be measured to determine if photosystem I and photosystem II structures and components are affected by the injury. Light-response curves determined in this study are not as precise as measurements with a fluorometer.

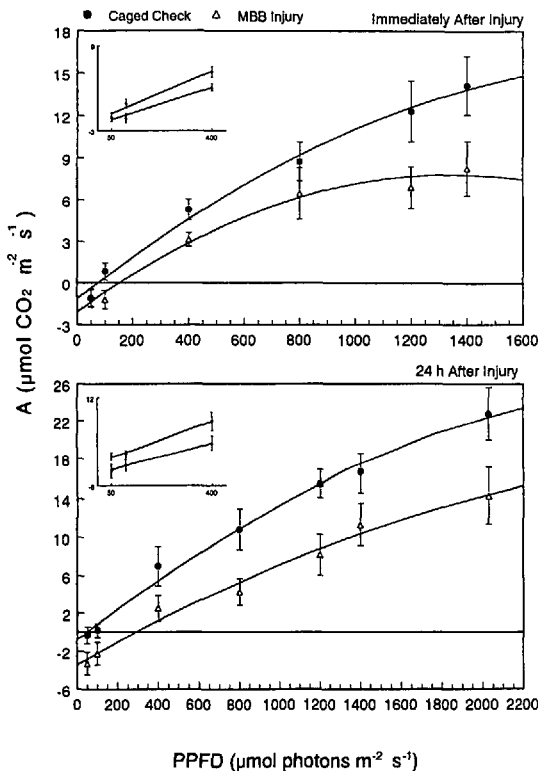


Fig. 8. The relationship between assimilation and photosynthetic photon flux density for injured and uninjured soybean leaflets (Clark 3W). Vertical, capped lines are standard error. Top graph represents measurements taken immediately after the cessation of injury; bottom graph represents measurements taken 24 h after the cessation of injury. Inset graphs are initial linear slopes representing quantum efficiencies. Data are from 1994.

Our findings indicate that Mexican bean beetle injury influences photosynthesis most likely through RuBP regeneration or utilization. The disruption of RuBP regeneration seems to be associated with alterations in the carbon reactions of the photosynthetic carbon reduction cycle. However, based on this research, we cannot determine precisely where the limitations are occurring.

Because injured tissue that is not consumed often turns necrotic and drops from the leaflet, it would be reasonable to hypothesize that herbivory by Mexican bean beetles reduces photosynthesis through disruption of leaflet water relations and vascular transport of assimilates. Mechanistically, reductions in photosynthesis would occur because of reductions in stomatal conductance and a build-up of Calvin cycle intermediates. Although this mechanism may operate in tissue isolated by feeding, our data indicate that stomatal conductances are following photosynthesis, rather than limiting photosynthesis. Moreover, reductions in photosynthesis occur across the entire leaflet, even in uninjured tissue at the basal portion of the leaflet near the midrib vein. This observation is supported by the finding that leaflets do not compensate for, or recover from, injury.

How this type of injury causes reductions in photosynthesis across the entire leaflet is unclear. Because the whole leaflet is affected, endogenous signals such as phytohormones may be involved, given that phytohormones have been implicated in rate limitations of photosynthesis (Sharkey 1985).

Alternately, cell wall fragments (specifically oligosaccharides) are known to act as wound signals in physiological responses to some pathogens (Fry et al. 1993). It is conceivable a similar signal transduction system might occur with skeletonizing injury, as from Mexican bean beetle feeding, resulting in altered leaflet gas exchange. Oligosaccharides are suspected to be involved in hypersensitive reactions of plants to some insect herbivores, although none of the existing examples involve skeletonizing leaf injury (Fernandes 1990).

Determining the potential mechanisms for photosynthetic rate limitations under any environmental stress (biotic or abiotic) is not simple, given the lack of understanding of many basic plant physiological processes associated with photosynthesis (Sharkey 1985). Nevertheless, determining mechanisms underlying rate reductions after arthropod injury is a crucial step in building more encompassing understandings of the physiology of biotic stress.

Physiological responses of soybean and dry bean to injury by Mexican bean beetle has not been determined at a level higher than the individual leaflet. Future research must address how plants respond to this injury type at these higher levels. Although individual leaflets do not compensate for injury, whole plants may compensate through interactions with various potential extrinsic and intrinsic factors. Extrinsic factors may include enhanced water status of remaining leaves, enhanced nutrient availability, or increased light penetration to leaflets on lower nodes (Welter

1989, Peterson et al. 1992). The latter mechanism seems likely, given the nature of Mexican bean beetle injury. Intrinsic factors may include increased assimilate demand after defoliation (Neales and Incoll 1968), reduced competition between leaves for mineral nutrients necessary for cytokinin production (Wareing et al. 1968), or delayed leaf senescence (Gifford and Marshall 1973, Caldwell et al. 1981, Nowak and Caldwell 1984). Given that soybean and dry bean compensate for other types of leaf injury (specifically leaf-mass consumption) through delayed leaf senescence, it seems likely this mechanism could operate with Mexican bean beetle injury. Ultimately, examination of whole plant photosynthetic responses to Mexican bean beetle is needed to address the question of compensation at the whole plant level.

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