Photosynthetic Responses of Wheat, *Triticum aestivum* L., to Defoliation Patterns on Individual Leaves

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ABSTRACT The impact of defoliation by fall armyworm, *Spodoptera frugiperda* (J. E. Smith), on the photosynthetic rates of injured, individual wheat, *Triticum aestivum* L., leaves and the impact of different spatial patterns of artificial insect defoliation on photosynthesis of remaining leaf tissue of injured, individual wheat leaves were evaluated in this study. Photosynthesis, stomatal conductance, transpiration, and chlorophyll *a* fluorescence were recorded in the flag-leaves of wheat plants 1 and 24 h after defoliation in 2003 and at 1 h, 24 h, 7 d, and 14 d after defoliation in 2004. Photosynthesis of injured leaves was not significantly affected by any defoliation treatment (i.e., control, natural, and artificial). Similarly, we did not observe interactions between defoliation treatments and time after defoliation. Stomatal conductance was significantly affected by time after defoliation and by the interaction between defoliation treatment and time after defoliation. However, in general, our results showed that wheat responded similarly to insect defoliation and artificial defoliation, which, therefore, may be used to simulate leaf mass consumption. Spatial defoliation patterns had a significant effect on photosynthetic parameters of injured leaves, but responses were dependent on plant developmental stages. The chlorophyll *a* fluorescence data revealed no significant effects from any defoliation pattern on the photochemical efficiency of the injured leaf. No significant interactions between defoliation patterns and time after defoliation were observed. Our findings reveal that the spatial pattern of defoliation in wheat affects photosynthetic and other gas exchange responses, which suggests that when simulating insect defoliation in wheat, researchers need to be cognizant of the defoliation pattern to adequately simulate insect defoliation.

KEY WORDS herbivory, *Spodoptera frugiperda*, photosynthesis, ecophysiology, plant gas exchange

Although ~25% of all animal species are represented by herbivorous terrestrial insects (Strong et al. 1984), the impact of herbivory on plants remains a controversial topic. Numerous studies have been conducted to better understand the effect of herbivorous insects, especially defoliators that are leaf-mass consumers, on plant photosynthetic metabolism (Poston et al. 1976, Hammond and Pedigo 1981, Ingram et al. 1981, Ostlie and Pedigo 1984, Welter 1991, Higley 1992, Peterson et al. 1992, 2004, Peterson and Higley 1996). Leaf-area reduction by defoliators can elicit either increases (Wareing et al. 1968, Gifford and Marshall 1973, Satoh et al. 1977, Aoki 1981, Von Caemmerer and Farquhar 1984, Baysdorfer and Bassham 1985, Williams and Farrar 1988, Tschaplinski and Blake 1989, Welter 1989, Layne and Flore 1992, 1993), transient decreases (Alderfelder and Eagles 1976, Hall and Ferree 1976, Li and Proctor 1984), or, perhaps more commonly, no long-term photosynthetic changes on remaining photosynthetically active tissue (Davidson and Milthorpe 1966, Poston et al. 1976, Syvertsen and McCoy 1985, Welter 1989, 1991, Higley 1992, Peterson et al. 1992, 1996, 2004, 2005, Peterson and Higley 1996, Burkness et al. 1999).

Possible explanations for these variable outcomes might be related to differences in study methodologies and foci. Although some of these studies determined the impact of herbivory on plant photosynthesis through whole plant assessments, others evaluated only the photosynthesis of individual leaves, making generalizations difficult (Peterson 2001). In addition, plant responses to insect injury are highly dependent on the time of injury with respect to plant phenology, intensity of injury, part of plant injured, type of injury (including spatial pattern of injury), and environmental factors (Pedigo et al. 1986, Higley et al. 1993, Peterson and Higley 2001, Macedo et al. 2005). Despite the identification of the factors described above, potential reasons for variable plant responses have not been systematically and comprehensively explored.

Artificial defoliation has been used widely to simulate the effects of herbivory on plant primary physiology, growth, and yield. Although there are limitations associated with the use of artificial defoliation (Baldwin 1990), studies have shown that artificial defoliation can properly simulate many plant responses

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(including photosynthesis) compared with actual insect defoliation (Detling et al. 1979, Boote et al. 1980, Buntin and Pedigo 1985, Welter 1991, Peterson et al. 1992, Burkness et al. 1999).

Despite the increasing number of studies to develop generalized models of plant physiological response to defoliation, most of the research has been conducted on relatively few plant species, such as soybean, *Glycine max* L. Merrill, and alfalfa, *Medicago sativa* L., (Poston et al. 1976, Hammond and Pedigo 1981, Ingram et al. 1981, Ostlie and Pedigo 1984, Higley 1992, Peterson et al. 1992, Peterson and Higley 1993, 1996, Peterson 2001, Peterson et al. 2004). Other than for detailed studies of several crop or fruit species, including apple, *Malus pumila* Mill. (Hall and Ferree 1976, Peterson et al. 1996), cotton, *Gossypium hirsutum* L. (Holman and Oosterhuis 1999), beans, *Phaseolus vulgaris* L. (Peterson et al. 1998), and, to a lesser degree, wheat, *Triticum aestivum* L. (Detling et al. 1979, Haile et al. 1999, Macedo et al. 2003, 2005, 2006a, 2006b), a basic understanding about how plants respond physiologically to insect defoliation is still needed. Peterson et al. (2004) argued that research on photosynthetic response to leaf mass consumption injury needs to occur for more plant–insect systems to better understand the strengths and limitations of generalized models of response.

There are studies with wheat in which the effects of defoliation have been characterized (Culy 2001, Macedo et al. 2006b). Most of the studies evaluated morphological, growth, and yield components, but wheat photosynthetic responses, either at whole plant or at leaf levels, have not been extensively studied. Consequently, the objectives of this study were to (1) determine the impact of simulated and actual defoliation by fall armyworm, *Spodoptera frugiperda* (J.E. Smith), on photosynthetic rates of injured, individual leaves, and (2) evaluate the impact of different spatial patterns of artificial insect defoliation on photosynthesis of remaining leaf tissue of individual, injured leaves. We used wheat as our plant system because, in a series of recent studies, we and others have been characterizing its physiological responses to several insect herbivore injury guilds (Burd et al. 1993, Miller et al. 1994, Budak et al. 1999, Haile et al. 1999, Heng-Moss et al. 2003, Macedo et al. 2003, Ni and Quisenberry 2003, Wang et al. 2004, Macedo et al. 2005, 2006a).

Materials and Methods

Plant Material and Experimental Conditions. Experiments were conducted in the Montana State University Plant Growth Center greenhouses, Bozeman, MT, during 2003. Spring wheat, variety McNeal, was grown in pots (13.3 by 13.3 by 14.6 cm) in a mixture of 'Sunshine' soil mix and sand mix (1:1 ratio) in a greenhouse bay (32 m^2). Plants were watered regularly and fertilized twice per week with a 100 ppm mix (Peters 20-20-20 General, Scotts-Sierra Hort. Prod. Company, Marysville, OH). Plants were maintained in the greenhouse bay at 21 ± 1°C, photoperiod of 14:10 h (L:D), and 40–50% RH for the duration of the study. To increase light quality/intensity inside the greenhouse, supplemental lighting, consisting of GE Multi-Vapor lamps (MVR1000/C/U, GE Lighting; General Electric Co., Cleveland, OH) was provided. The light intensity in the greenhouse at the canopy level, under a clear sky at midday, was 970 μmol photons/m^2/s, recorded during photosynthetic measurements using a quantum sensor (model LI-190; LI-COR, Lincoln, NE).

Insect Versus Artificial Defoliation. The experimental design consisted of a randomized complete block design (RCBD) with five replications per treatment, blocked by light source, as described above. Treatments consisted of a no defoliation control, insect defoliation, and artificial defoliation treatments. The treatments were imposed on the most recent fully expanded leaf of the elongating primary stem. Third and fourth instars of *S. frugiperda*, previously fed on artificial diet, were placed on a leaf and allowed to feed until the desired defoliation levels of ≈30–60% were reached (<4 h) on plants at the stem elongation stage, Zadoks 30–32 (Zadoks et al. 1974). Larvae that departed from the marked, experimental leaf were either returned to it or were replaced by new larvae. Artificial defoliation was imposed by using a pair of scissors at the same time the larvae were feeding. Approximately 50% of leaf tissue was removed in a pattern consistent with *S. frugiperda* feeding. Two experimental replications were conducted. Percentage of leaf tissue removal on each leaf by *S. frugiperda* was visually estimated.

Spatial Patterns of Artificial Defoliation. The experimental design consisted of an RCBD with five treatment replications, blocked by light source. To impose treatments, five different defoliation patterns (control, 50% distal excisions with remaining basal tissue, 50% basal excisions with remaining distal tissue, 25% distal excisions, and 25% basal; Fig. 1A) were imposed on leaves at seedling, tillering, stem elongation, and boot/flowering developmental stages (Zadoks 14–16, 24–26, 32, 49–57), using a pair of scissors. Treatments were imposed on the most recent fully expanded leaf of the elongating primary stem. Each developmental stage was assessed with the following experimental replications: seedling ($n = 3$) for a total of 15 replications per treatment, tillering ($n = 1$) for a total of five replications per treatment, stem elongation ($n = 1$) for a total of five replications per treatment, and boot/flowering ($n = 2$) for a total of 10 replications per treatment.

Based on our 2003 results, four defoliation patterns (control, 50% distal excisions, 50% basal excisions without remaining tissue, and 50% middle section excisions; Fig. 1B), were imposed in 2004 on leaves at the seedling developmental stage, Zadoks 14–16, using a pair of scissors. Treatments were imposed on the most recent fully expanded leaf of the elongating primary stem. Two experimental replications were conducted.

Photosynthetic Measurements. We measured the photosynthetic capacity of all experimental plants. Photosynthesis and closely associated processes, such as transpiration, stomatal conductance, and intercel-

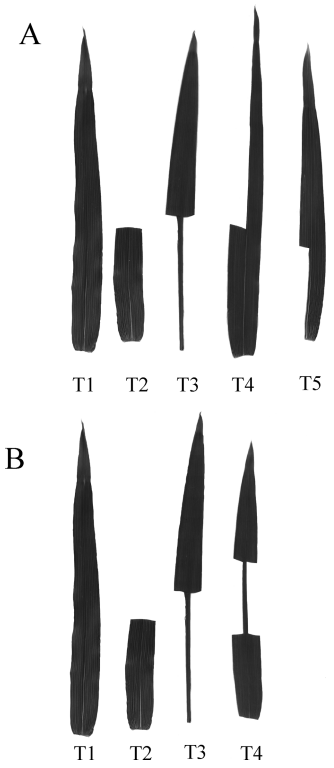

Fig. 1. Spatial patterns of defoliation: (A) 2003 defoliation patterns (control: T1, 50% distal excisions with remaining basal tissue: T2, 50% basal excisions with remaining distal tissue: T3, 25% distal excisions: T4, and 25% basal: T5) and (B) 2004 defoliation patterns (control: T1, 50% distal excisions: T2, 50% basal excisions without remaining tissue: T3, and 50% middle section excisions: T4).

lular CO_2 rates were recorded from the injured leaves using a portable photosynthesis system (model LI-6400; LI-COR) at 1,200 μmol photons/m^2/s light intensity and 400 μmol/mol CO_2 reference concentration at a constant flow of 500 μmol/s. Data were recorded when the system was considered stable (i.e., photosynthesis changes were <0.1 μmol/m^2/s, and conductance changes were <0.05 μmol/m^2/s).

Additionally, in 2004, chlorophyll *a* fluorescence measurements were recorded from a subset of plants within each treatment ($n = 3$) on the injured leaf using a leaf-chamber fluorometer (model LI-6400–40; LI-COR, Lincoln, NE). We performed a kinetic test to determine the photochemical efficiency of photosystem II. The parameters measured were nonvariable fluorescence, the overall photochemical quantum yield, the apparent photosynthetic electron transfer rate, and the quenching coefficients, nonphotochemical quenching, and photochemical quenching. Chlorophyll *a* kinetics were measured at 400 μmol/mol CO_2 concentration, 1,200 μmol photons/m^2/s light intensity, measuring intensity 1 Int, measuring modulation 0.25 kHz, measuring filter 1 Hz, measuring gain 10 Gn, flash duration 0.8 s, flash intensity 7 Int, flash modulation 20 kHz, and flash filter 50 Hz settings.

In 2003, photosynthetic parameters were measured from injured leaves 1 and 24 h after each defoliation event. In 2004, all photosynthetic and chlorophyll *a* fluorescence measurements were taken from injured leaves 1 h, 24 h, 7 d, and 14 d after each defoliation event.

Statistical Analysis. Analysis of variance (ANOVA) procedures were performed to determine the difference variances for multiple experimental replications of *S. frugiperda* versus artificial defoliation experiments by including trials in the ANOVA model using PROC MIXED procedure (SAS Institute 2001). The overall ANOVA model included main effects as defoliation treatments (i.e., nondefoliation, insect defoliation, and artificial defoliation), experimental replication, time (i.e., hours and/or days after each defoliation treatment), and their interactions. Blocks were assumed to have random effects in the model. Data were pooled when interactions between experiment and treatments were not significant. When appropriate, means were separated using pairwise least-squares means (LSMEANS) procedure ($\alpha = 0.05$).

The same statistical approach was used to determine the effects of artificial defoliation patterns impact on plant physiological response. To determine the short- and long-term impact of different defoliation patterns on the parameters of interest, data were analyzed using repeated measures (PROC MIXED; SAS Institute 2001). The overall ANOVA model included main effects as defoliation treatments (control, 50% distal excisions with remaining basal tissue, 50% basal excisions with remaining distal tissue, 25% distal excisions, and 25% basal), experimental replication (when appropriate), time (i.e., hours and/or days after each defoliation treatment), and their interactions for each plant developmental stage. Blocks were assumed to have random effects in the model. When appropriate, means were separated using pairwise least-squares means (LSMEANS) procedure ($\alpha = 0.05$).

Results and Discussion

Insect Versus Artificial Defoliation. Our ANOVA results indicated that the interactions between experimental replication and treatments were not significant ($F = 2.21$; df = 2,19; $P = 0.14$); therefore, there were 10 replications per treatment (two experimental replications $\times$ 5 treatment replications) in our analysis.

Feeding by *S. frugiperda* larvae resulted in mean defoliation levels of 37.6 $\pm$ 5.2% (SEM). Photosynthesis of injured leaves was not significantly affected by any of the defoliation treatments (i.e., control, natural, and artificial; $F = 0.46$; df = 2,12; $P = 0.94$). Similarly, we did not observe interactions between defoliation treatments and time after defoliation ($F = 1.22$; df = 2,39; $P = 0.31$). Conversely, we observed a significantly greater stomatal conductance caused by defoliation and its interaction with time after defoliation on remaining leaf tissue of injured leaf ($F = 4.54$; df = 2,39; $P = 0.02$). We observed significantly greater stomatal conductance values 24 h after defoliation compared with 1 h for *S. frugiperda* defoliated leaves (Table 1). Higher stomatal conductance was also ob-

Table 1. Mean ± SEM values for the impact of defoliation type (i.e., control, *S. frugiperda*, and artificial) on remaining leaf tissue photosynthesis (Ps), stomatal conductance (g_s), intercellular CO_2 (C_i), and transpiration (E) measured 1 and 24 h after defoliation

Photosynthetic parameters	Defoliation					
	Control		*S. frugiperda*		Artificial	
	1 h	24 h	1 h	24 h	1 h	24 h
Ps (μmolCO_2/m^2/s)	18.41 ± 3.3*a*	17.22 ± 1.2*a*	17.54 ± 2.9*a*	18.85 ± 3.3*a*	19.19 ± 5.6*a*	17.28 ± 3.3*a*
g_s (mol H_2O/m^2/s)	0.32 ± 0.07*a*	0.41 ± 0.03*a*	0.33 ± 0.05*a*	0.56 ± 0.07*b*	0.35 ± 0.13*a*	0.43 ± 0.09*a*
C_i (μmol CO_2 mol/air)	276.7 ± 24.4*a*	306.0 ± 11.7*a*	287.0 ± 21.5*a*	319.4 ± 12.9*a*	281.9 ± 18.1*a*	308.6 ± 14.4*a*
E (mol H_2O/m^2/s)	5.4 ± 1.6*a*	6.0 ± 0.8*a*	5.5 ± 0.8*a*	7.5 ± 0.9*a*	5.9 ± 2.17*a*	6.0 ± 0.7*a*

Means ± SEM followed by same letters within rows are not significantly different at $\alpha = 0.05$.

served on *S. frugiperda* defoliated leaves 24 h after defoliation compared with control and artificially defoliated leaves. Neither defoliation treatment nor its interaction with time after defoliation had a significant effect on intercellular CO_2 and transpiration of injured leaves (Table 1).

Our results showed that defoliation by *S. frugiperda* larvae did not affect photosynthesis of the remaining tissue of injured leaves. However, stomatal conductance values were higher, which may indicate that, shortly after defoliation was imposed, injured leaves had not closed their stomata. In addition, the lack of significant effects on other photosynthetic parameters such as intercellular CO_2 and transpiration suggests that water loss was not a limiting factor for photosynthesis in the injured leaves for at least 24 h after defoliation. Previous studies have shown that artificial defoliation can be used to mimic certain plant physiological, developmental, and yield/fitness effects of leaf mass reduction as a result of insect or vertebrate herbivory (Detling et al. 1979, Boote et al. 1980, Buntin and Pedigo 1985, Welter 1991, Peterson et al. 1992, 2004, 2005, Burkness et al. 1999). Our results also showed that, despite transient higher stomatal conductance, wheat responds similarly to actual insect defoliation and artificial defoliation, and therefore, may be used to simulate leaf mass consumption. Additionally, our results provided additional evidence supporting that leaf mass consumption might only cause short-term photosynthetic changes on remaining photosynthetically active tissue (Davidson and Milthorpe 1966, Poston et al. 1976, Syvertsen and McCoy 1985, Welter 1989, 1991, Higley 1992, Peterson et al. 1992, 1996, 2005, Peterson and Higley 1996, Burkness et al. 1999). However, our data are limited to a particular developmental stage (i.e., stem elongation). It is still not clear whether similar plant responses would be observed at different developmental stages, such as seedlings, reproduction, maturation, etc.

Spatial Patterns of Artificial Defoliation. Our 2003 data indicated that there was no significant interaction between experimental replications and treatment replications for any of the developmental stages evaluated: stages 14–16 (photosynthesis: $F = 0.74$, df = 8,125, $P = 0.66$; stomatal conductance: $F = 1.68$, df = 8,125, $P = 0.11$; intercellular CO_2: $F = 0.61$, df = 8,125, $P = 0.76$; transpiration: $F = 0.27$, df = 8,125, $P = 0.97$); stages 24–26; stage 32; and stages 49–57 (photosynthesis: $F = 0.98$, df = 4,75, $P = 0.42$; stomatal conductance: $F = 1.29$, df = 4,75, $P = 0.28$; intercellular CO_2: $F = 0.27$, df = 4,75, $P = 0.89$; transpiration: $F = 1.46$, df = 4,75, $P = 0.22$). Therefore, to determine the impact of defoliation patterns on photosynthetic response of injured leaves of each developmental stage, the data from each trial were pooled.

We did not observe significant interactions between defoliation pattern and time (i.e., 1 and 24 h after defoliation) for plants at any developmental stage. Leaves defoliated at the basal portion, both 50 and 25%, had significantly lower photosynthesis (Table 2).

We observed significantly lower photosynthetic rates on leaves 50% defoliated at the basal portion of plants at seedling, tillering, and boot/flowering developmental stages (Table 2). We also observed similar photosynthetic reductions on leaves treated with 25% basal excisions at seedling and tillering developmental stages (Table 2).

In general, lower photosynthesis rates were accompanied by lower stomatal conductance and transpiration rates. No significant alterations in intercellular CO_2 were observed (Table 2).

Conversely, both basal excisions (i.e., 50 and 25% basal excisions), did not affect leaf photosynthesis of plants at stem elongation and boot/flowering developmental stages. However, lower stomatal conductance was observed on leaves treated with 50% basal excision on plants at stem elongation stage. Similarly, lower stomatal conductance was observed on leaves of plants at boot/flowering developmental stages treated with both 50 and 25% basal excisions (Table 2).

Similar to results obtained in 2003, our 2004 data indicated that there were no significant interactions between experimental replications and treatment replications. Therefore, to determine the impact of defoliation patterns on photosynthetic capacity of injured leaves of each developmental stage, the data from each experiment were pooled.

We did not observe any significant interaction between patterns of defoliation and time after defoliation on photosynthesis of injured leaves ($F = 1.92$, df = 9,47, $P = 0.07$). However, photosynthesis, stomatal conductance, and transpiration of leaves from all defoliation patterns were significantly different as plant development proceeded over the 14 d (Table 3). Defoliation patterns alone significantly impaired photosynthesis of injured leaves ($F = 26.33$, df = 3,16, $P < 0.0001$; Table 3). These photosynthesis differences were accompanied by significant lower stomatal con-

Table 2. Mean ± SEM values from 2003 experiments on the impact of different spatial patterns of artificial insect defoliation on photosynthesis (Ps), stomatal conductance (g_s), intercellular CO_2 (C_i), and transpiration (E) of remaining leaf tissue of wheat plants at four developmental stages

Plant stage	Parameter	Defoliation pattern				
		Control	Distal 50%	Basal 50%	Distal 25%	Basal 25%
Seedling (Zadoks 14–16)	Ps (μmolCO_2/m^2/s)	26.41 ± 5.3*a*	27.58 ± 5.3*a*	18.24 ± 7.64*b*	26.44 ± 4.73*a*	21.34 ± 6.49*b*
	g_s (mol H_2O/m^2/s)	0.38 ± 0.18*a*	0.41 ± 0.17*a*	0.26 ± 0.14*b*	0.39 ± 0.16*a*	0.31 ± 0.16*ab*
	C_i (μmol CO_2 mol/air)	274.6 ± 69.6*a*	280.9 ± 70.6*a*	274.5 ± 93.3*a*	287.9 ± 57.2*a*	277.1 ± 84.6*a*
	E (mol H_2O/m^2/s)	8.47 ± 2.8*a*	9.06 ± 2.9*a*	6.52 ± 3.2*b*	8.80 ± 2.5*a*	7.50 ± 2.8*b*
Tillering (Zadoks 24–26)	Ps (μmolCO_2/m^2/s)	20.24 ± 3.7*a*	18.84 ± 2.8*a*	9.33 ± 3.6*b*	19.28 ± 5.13*a*	14.13 ± 3.5*c*
	g_s (mol H_2O/m^2/s)	0.36 ± 0.15*a*	0.37 ± 0.14*a*	0.18 ± 0.09*b*	0.33 ± 0.16*a*	0.24 ± 0.09*b*
	C_i (μmol CO_2 mol/air)	306.3 ± 45.2*a*	318.3 ± 45.2*a*	326.8 ± 43.5*a*	304.7 ± 42.4*a*	308.2 ± 46.8*a*
	E (mol H_2O/m^2/s)	8.98 ± 1.1*a*	9.31 ± 1.5*a*	5.60 ± 1.1*b*	8.68 ± 2.2*a*	6.99 ± 1.4*b*
Stem elongation (Zadoks 32)	Ps (μmolCO_2/m^2/s)	22.7 ± 3.6*a*	22.3 ± 5.0*a*	17.7 + 5.6*a*	21.2 ± 4.4*a*	21.9 ± 2.4*a*
	g_s (mol H_2O/m^2/s)	0.35 ± 0.07*a*	0.33 ± 0.09*a*	0.22 ± 0.07*b*	0.33 ± 0.08*a*	0.29 ± 0.05*a*
	C_i (μmol CO_2 mol/air)	306.8 ± 12.9*a*	302.3 ± 11.6*a*	291.3 ± 45.8*a*	308.2 ± 21.6*a*	292.8 ± 21.8*a*
	E (mol H_2O/m^2/s)	6.73 ± 1.2*a*	6.32 ± 1.7*a*	4.81 ± 1.1*b*	6.39 ± 1.2*a*	6.09 ± 1.0*a*
Boot/flowering (Zadoks 49–57)	Ps (μmolCO_2/m^2/s)	23.77 ± 3.7*a*	22.86 ± 2.4*a*	15.32 ± 4.7*b*	23.50 ± 4.4*a*	19.56 ± 3.6*a*
	g_s (mol H_2O/m^2/s)	0.38 ± 0.08*a*	0.37 ± 0.08*a*	0.22 ± 0.08*b*	0.36 ± 0.12*a*	0.29 ± 0.07*c*
	C_i (μmol CO_2 mol/air)	301.2 ± 24.2*a*	299.1 ± 23.9*a*	297.9 ± 27.3*a*	290.3 ± 28.1*a*	300.3 ± 32.8*a*
	E (mol H_2O/m^2/s)	8.1 ± 1.6*a*	7.7 ± 1.6*a*	5.6 ± 1.8*b*	7.8 ± 2.1*a*	7.1 ± 1.6*a*

Means ± SEM followed by same letters within rows are not significantly different at $\alpha = 0.05$.

ductance and transpiration values for injured leaves. Similarly, transpiration of 50% distal excision treated leaves were 85% higher than the observed on 50% basal excision treated leaves ($t = 4.20$, df = 47, $P = 0.0001$; Table 3). No significant differences were observed on the other defoliation patterns. No significant differences were observed in intercellular CO_2 among different defoliation patterns at any data collection date (Table 3).

The chlorophyll *a* fluorescence data revealed no significant effects of any defoliation pattern on the photochemical efficiency of the injured leaf. No significant interactions between defoliation patterns and time after defoliation were observed.

Our results suggest that photosynthesis of injured leaves was affected differently depending on the spatial defoliation pattern. In general, photosynthetic impairment was observed on leaves with basal defoliation patterns (i.e., 50 and 25% basal excisions in 2003 and 50% basal excision in 2004). Defoliation patterns in which leaf area reductions were imposed on the basal portion had lower photosynthesis, stomatal conductance, and transpiration rates that indicate that stomatal limitations might be directly related to impairment of the photosynthetic capacity of injured leaves. It also might relate to the monocotyledonous leaf venation running parallel from the base to the apex of the leaf; therefore, the translocation of water and photoassimilates might be variably disrupted by the tested defoliation patterns. It is possible that end-products accumulate in some portions of the leaf, resulting in inhibition of photosynthesis. Consequently, to maintain leaf homeostasis, reductions of CO_2 uptake and assimilation would occur, resulting in the observed stomatal closure, leading to lower stomatal conductance and transpiration.

Alternatively, the observation of impaired photosynthesis in leaves exposed to this specific defoliation pattern (i.e., basal defoliation) might be solely because the leaf's most photosynthetically active section was removed, leaving the most mature and less active portion because of the senescence processes. Haile et al. (1999) observed different photosynthetic activity in different portions of wheat leaves. They observed a

Table 3. Mean ± SEM values from 2004 experiments for the impact of different spatial patterns of artificial insect defoliation on photosynthesis (Ps), stomatal conductance (g_s), intercellular CO_2 (C_i), and transpiration (E) of remaining leaf tissue of seedling wheat plants (growth stage 14–16) measured 1 h, 24 h, 7 d, and 14 d after defoliation

Photosynthetic parameters	Defoliation patterns			
	Control	Distal 50%	Basal 50%	Middle 50%
Ps (μmolCO_2/m^2/s)	16.1 ± 0.8*a*	19.7 ± 0.8*b*	9.8 ± 0.83*c*	13.5 ± 0.81*d*
g_s (mol H_2O/m^2/s)	0.12 ± 0.07*a*	0.19 ± 0.08*b*	0.05 ± 0.03*c*	0.09 ± 0.06*a*
C_i (μmol CO_2 mol/air)	172.8 ± 80.1*a*	196.6 ± 71.4*a*	231.3 ± 178.1*a*	177.0 ± 107.3*a*
E (mol H_2O/m^2/s)	2.24 ± 1.5*a*	3.19 ± 1.9*b*	0.94 ± 0.7*c*	2.07 ± 1.8*a*
	Time after defoliation			
	1 h	24 h	7 d	14 d
Ps (μmolCO_2/m^2/s)	16.9 ± 4.5*a*	20.7 ± 4.85*a*	15.7 ± 3.9*b*	9.4 ± 0.8*c*
g_s (mol H_2O/m^2/s)	0.12 ± 0.05*a*	0.16 ± 0.06*a*	0.05 ± 0.04*b*	0.09 ± 0.01*a*
C_i (μmol CO_2 mol/air)	145.9 ± 38.2*a*	150.7 ± 51.6*a*	161.5 ± 130.7*a*	245.6 ± 22.4*ab*
E (mol H_2O/m^2/s)	3.95 ± 1.6*a*	2.87 ± 0.9*b*	0.91 ± 0.6*c*	1.09 ± 0.18*c*

Means ± SEM followed by same letters within rows are not significantly different at $\alpha = 0.05$.

decline in leaf photosynthetic activity based on base-to-apex measurements along the leaf. Because we simulated possible defoliation patterns imposed by different herbivorous insects, it was necessary to measure photosynthesis in slightly different portions of the leaves, which may explain the observed results.

A major event in the leaf senescence process is the disassembly of the photosynthetic apparatus (i.e., detachment of the antennal chlorophyll complex from PSII), which would translate into impairment of photosystem II photochemistry. However, in this study, the lack of significant effects of any defoliation treatments on any of the chlorophyll *a* fluorescence parameters measured indicates that changes in photosynthesis of injured leaves most likely were related solely to the different spatial defoliation patterns.

Our results revealed that the spatial pattern of defoliation in wheat affects photosynthetic and other gas exchange responses, which is interesting because Piesik et al. (2006) showed that volatile secondary metabolite production also is altered for varying spatial patterns of artificial defoliation that are similar to the ones used in this study. Piesik et al. (2006) performed their study using the same wheat variety under the same growing conditions as this study, but for a different year. Similar patterns for primary metabolic responses have not been observed in soybeans, where different spatial patterns of injury on individual leaves did not affect primary physiological responses (Poston et al. 1976, Hammond and Pedigo 1981, Ostlie and Pedigo 1984, Peterson and Higley 1996). This most likely is because of differences in leaf development and morphology between wheat and soybean.

Our results suggest that, when simulating insect defoliation in wheat, researchers need to be cognizant of the defoliation pattern and not simply assume that clipping the distal half of each leaf, for example, will adequately simulate insect defoliation. Although the spatial pattern of wheat defoliation is important, when simulating insect defoliation from larger species, such as later instar lepidopterans and grasshoppers, it is probably of much less concern.

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