Wheat Stem Sawfly, *Cephus cinctus* Norton, Impact on Wheat Primary Metabolism: An Ecophysiological Approach

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ABSTRACT The impact of wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), on the photosynthetic capacity and primary metabolism of wheat, *Triticum aestivum* L., was evaluated in three different environments: environmental growth chamber, greenhouse, and field. *C. cinctus* elicited different photosynthetic responses in different environments. Wheat gas exchange parameters, such as photosynthesis, stomatal conductance, intercellular CO_2, and transpiration in the growth chamber environment were negatively affected by *C. cinctus* feeding. Conversely, the same gas exchange responses were not observed under greenhouse and field conditions. This study shows the important role of environmental variables, such as ambient CO_2 concentrations and light intensity, on plant responses to herbivores.

KEY WORDS *Triticum aestivum*, herbivory, photosynthesis, eco-physiology, injury guilds

INJURY BY MANY HERBIVOROUS arthropod species has long been known to have a negative impact on plant photosynthesis and yield in both agricultural and natural systems (Welter 1989, Peterson and Higley 1993, Delaney and Macedo 2001, Peterson 2001). Characterizing primary metabolic mechanisms, such as gas exchange, underlying plant responses to arthropod injury is needed to adequately explain fitness and yield loss and develop general models of plant response (Peterson 2001, Peterson and Higley 2001). Plant gas exchange processes, such as photosynthesis, water-vapor transfer, and respiration, represent a subset of a plant's primary metabolic processes. Consequently, understanding how arthropod injury influences these parameters is important because these are the primary processes determining plant growth, development, and, ultimately, fitness (Peterson and Higley 1993). Furthermore, these processes respond very rapidly to external factors, so their measurement provides an immediate indication of plant stress (Peterson and Higley 1996, Peterson et al. 1998, Macedo et al. 2003).

Unfortunately, the physiological mechanisms underlying plant responses to arthropod injury are still poorly understood (Peterson and Higley 1993, 2001). Progress in understanding plant physiological responses to arthropod injury resulted primarily from a consideration of injury types. Boote (1981), Pedigo et al. (1986), and Higley et al. (1993) emphasized the use of categorizing plant biotic stressors based on injury type and response rather than on the taxonomic classification of stressors or physical appearance of injury (as traditionally had been done). Furthermore, Peterson and Higley (2001) argued that similarities in plant response to specific injury types (also known as injury guilds) can be used effectively to address many basic and applied research questions.

The most complete studies of arthropod injury and plant gas exchange responses involve the leaf-mass consumption (defoliation) injury guild (Peterson 2001, Peterson et al. 2004). Other injury guilds, such as assimilate sapping, mesophyll feeding, and leaf mining, have been studied much less. Virtually nothing is known about plant physiological responses to the stem-boring injury guild.

Heichel and Turner (1973) and Madden (1977) suggested that stem boring impaired photosynthetic capacity primarily because of injury to vascular tissue. Godfrey et al. (1991) observed reductions in photosynthesis of corn injured by European corn borer, *Ostrinia nubilalis* (Hübner). In particular, they noted 10–20% photosynthetic rate reductions associated with larval stem boring and concluded that the reductions may have been related to alterations of source-sink relationships. Rubia et al. (1996) observed compensatory mechanisms leading to an increase in photosynthesis of borer-injured rice tillers.

Plant primary metabolic responses to the wheat stem sawfly, *Cephus cinctus* Norton, a larval stem borer, have not been examined. *C. cinctus* is considered the most important insect pest of dryland wheat in the northern Great Plains. In Montana alone, losses by *C. cinctus* are approximately U.S. $25 million per year. To date, most of the research surrounding *C. cinctus* is directed at aspects of detection and management.

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However, understanding photosynthetic responses of wheat to *C. cinctus* also is of considerable practical and theoretical interest.

In Montana, *C. cinctus* adults emerge from late May to late June, but emergence may continue until the third week of July (Criddle 1915, 1923, Wallace and McNeal 1966, Weiss et al. 1990, Weiss and Morrill 1992, Morrill and Kushnak 1996), which is synchronized with host plant developmental stage that is suitable for *C. cinctus* oviposition (developmental stage 32–69) (Zadoks et al. 1974). Generally, the uppermost internodes of an elongating stem are preferred for oviposition (Holmes and Peterson 1960). Each female typically lays only one egg per stem (Ainslie 1920), but egg laying by more than one female per stem is common.

Larval development may include four or five instars (Ainslie 1920), and at the onset of plant maturation, larvae chew a notch around the inside perimeter of the stem near the soil level. Infested stems usually lodge, and the broken stems are highly visible to farmers during harvest (Ainslie 1920). Neonate larvae initially feed on parenchyma tissue near the oviposition site (Holmes 1954). Developing larvae disperse throughout the stems, and stem nodes are injured during the movement. Disruption of vascular tissues that are constricted at the nodes can result in an accumulation of material that appears as darkened areas below nodes (Morrill et al. 1992b). Stems are filled with "frass," consisting of fecal material and chewed plant tissue. Estimation of the reduction of grain resulting from larval feeding is complicated because ovipositing females prefer the largest stems that have the potential to produce comparatively more grain (Morrill et al. 1992a). However, estimates of loss range up to 25% (Morrill et al. 1992a).

The objective of this study was to characterize the impact of injury by the stem borer, *C. cinctus*, on primary metabolism of wheat. To do this, we conducted studies during 2002, 2003, and 2004 in three different environment settings: growth chamber, greenhouse, and field.

Materials and Methods

Study 1. In 2002 (4 June to 19 July), we conducted a growth chamber experiment at the Plant Growth Center, Montana State University-Bozeman. Spring wheat, 'McNeal', was grown in 10.15-cm pots in a mixture of Sunshine soil mix and sand mix (1:1 ratio) in a environmental growth chamber (Conviron, Winnipeg, Canada) under a photoperiod of 14:10 (L:D) h. Plants were watered regularly and fertilized twice per week with a 100-ppm mix (Peters 20–20-20 General, Scotts, Marysville, OH). To infest plants, four large Plexiglas cages (95 by 46 by 46 cm), each containing four pots of wheat at developmental stage 32 (Zadoks et al. 1974), were infested with 20 male and 20 female *C. cinctus* adults. Insects were allowed to mate and oviposit freely for a period of 7 d, after which plants were removed from the cages and arranged in a randomized complete block design (RCBD) with four replicates. The treatment design consisted of two treatments: noninfested wheat (control) and *C. cinctus*–infested wheat (infested). Experimental units consisted of individual pots, each containing four wheat plants. The plants and *C. cinctus* were maintained in environmental growth chambers (Conviron) at 21 ± 1°C, photoperiod of 14:10 (L:D) h, with a light intensity of 63 μmol photons/m^2/s at plant level and 40–50% RH for the duration of the study. Supplemental light consisted of fluorescent lamps installed at a 3 Cool White:1 Gro-Lux ratio (F96T12/CW/1500, F72T12/CW/1500, F96T12/GRO/VHO, and F72T12/GRO/VHO; Sylvania Lighting Center, Dancers, MA).

Gas exchange parameters, such as photosynthesis (Photo), transpiration (E), and stomatal conductance (g_s) rates were recorded from one leaf, flag-leaf, on each plant using a portable photosynthesis system (model LI-6400; Licor, Lincoln, NE) at 1,200 μmol photons/m^2/s light intensity, 400 μmol/mol CO_2 concentration, and a constant flow of 500 μmol/s. A smaller subset of leaves (i.e., four leaves/treatment) was used to determine CO_2 response curve (A-C_i) and light response curve. A-C_i response curves were performed at constant light intensity (1,500 μmol photons/m^2/s) and varying the intercellular CO_2 (C_i) concentration at 50, 100, 200, 300, 400, 600, 800, and 1,000 μmol/mol.

Light-response curves or photosynthetic photon flux density (PPFD) response curves were performed at constant intercellular CO_2 (C_i) concentration (400 μmol/mol CO_2) and constant flow (500 μmol/s) and varying light intensity at 2,000, 1,500, 1,000, 500, 200, 100, 50, 20, and 0 μmol photons/m^2/s. Plant gas exchange parameters were measured weekly, 17, 24, 31, 38, and 45 d after removal of the plants from the cages. Responses of A to PPFD and responses of assimilation (A) to internal CO_2 concentration (C_i) were analyzed for each treatment by nonlinear regression (Layne and Flore 1995). The best-fit curve was evaluated by analysis of r^2. The CO_2 compensation point (Γ) was extrapolated as the C_i at which assimilation was 0.0 μmol/m^2/s. Estimated carboxylation efficiency (k) was calculated as the slope in the linear portion of the A-C_i response curve. Ribulose-1,5-bisphosphate ($RuBP_2$) regeneration rate limitations were expressed by a reduced assimilation at saturating CO_2 concentrations (Farquhar and Sharkey 1982). Carboxylation efficiencies from assimilation versus C_i were determined through linear regression analysis. Homogeneity of the independent regression was evaluated by Student's *t*-test (Steele and Torrie 1980).

Study 2. In the summers of 2003 and 2004, we conducted a total of three greenhouse experiments at the Plant Growth Center, Montana State University-Bozeman. Spring wheat, 'McNeal', was grown, watered, and fertilized following procedures described in study 1. To infest plants in the first (13 May to 23 June 2003) and second (16 May to 20 June 2003) experiments, four large cages (95 by 46 by 46 cm), each containing four pots of wheat at developmental stage 32 (Zadoks et al. 1974), were infested with 20 male and 20 female *C. cinctus* adults. Insects were allowed to mate

and oviposit freely for a period of 7 d, after which plants were removed from the cages and arranged in a RCBD with four replicates. The treatment design consisted of two treatments: noninfested wheat (control) and *C. cinctus*–infested wheat (infested). Experimental units consisted of individual pots, each containing four wheat plants. The plants and sawfly were maintained in a greenhouse bay at 21 ± 1°C, photoperiod of 14:10 (L:D) h, and 40–50% RH for the duration of the study. The light intensity in the greenhouse at plant level was ≈334 μmol photons/m^2/s during the experiment. Light supplement consisted of GE Multi-Vapor lamps (MVR1000/C/U; GE Lighting, General Electric Co., Cleveland, OH). Gas exchange measurements were recorded 14, 21, 29, and 35 d after plant removal from the cages in the first experiment and 14, 21, 28, and 35 d after plant removal form the cages in the second experiment, and consisted of the same measurements and settings described in the study 1. To infest plants in the third experiment (30 July to 21 September 2004), we used the same cages as in the previous experiments, but there were only two plants per cage at developmental stage 39 (Zadoks et al. 1974). Twenty-two females and four males were allowed to mate and oviposit freely for a period of 7 d. After the infestation period, plants were removed from the cages and arranged in a completely randomized design (CRD) with six replicates. Gas exchange measurements were recorded 17 and 22 d after the infestation period.

Study 3. The study was conducted during June and July 2004 in a field located in Gallatin County, MT. Spring wheat, 'McNeal', was grown in 0.30-m-wide row at ≈494,000 plants/ha in a Broko silt loam (0–4% slopes) according to the soil survey of Gallatin County Area, MT (USDA-NRCS). The field was maintained with standard agronomic practices for western Montana. An individual plot was three rows by 15 m in length. The distance between plots was 152 m. The study had five replications, consisting of two infestation treatments (infested and noninfested). Experimental plots were naturally infested by *C. cinctus* that had emerged in a fallow wheat field located near the plots and had immigrated into the crop during the wheat stem elongation period (June–July).

We determined *C. cinctus* presence/absence in wheat plants (infested or noninfested), levels of infestation (no. of infested stems/no. total of stems), insect developmental stages (egg, larva), and levels of injury (no. of damaged nodes) by randomly harvesting 25 plants/plot ($n = 75$) and splitting all the stems weekly using a X-ACTO knife (Hunt, Statesville, NC) during the total period of the experiment (17 June to 30 July).

Measures of the impact of *C. cinctus* injury on wheat primary metabolism were obtained by determinations of impairment on photosynthetic capacity after we observed larval feeding in the plant material. Photosynthetic parameters were measured with a portable photosynthesis system (model LI-6400; Licor). Photosynthetic rate (Photo), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were measured at 400 μmol/mol CO_2 concentration and 1,200 μmol photons/m^2/s light intensity.

To measure chlorophyll a fluorescence, a leaf chamber fluorometer (model LI-6400–40; Li-Cor) was used. We performed a kinetic test with the primary objective of determining the photochemical efficiency of photosystem II (PSII) in determining all fluorescence parameters, such as the nonvariable fluorescence (F_o), the overall photochemical quantum yield (Y), the apparent photosynthetic electron transfer rate (ETR), and the quenching coefficients—nonphotochemical quenching (qN), and photochemical quenching (qP). Chlorophyll *a* kinetics were measured at 400 μmol/mol CO_2 concentration, 1,200 μmol photons/m^2/s light intensity, with 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. Such measurements will indicate the overall photochemical efficiency of PSII.

All measurements were taken from the flag leaves on the primary stem and a tiller (i.e., two measurements/plant) during wheat reproductive and maturation stages, comprised of growth stages 49–87 (Zadoks et al. 1974).

Data for studies 1 and 2 were analyzed using repeated measures (PROC MIXED; SAS Institute 2001). For study 3, data were analyzed as a 2 by 2 factorial using PROC MIXED procedure of the SAS program (SAS Institute 2001). Means were separated by *t*-test ($\alpha = 0.05$).

Results

Study 1. We measured a total of 23 plants (7 infested and 16 control) starting 17 d after infestation. Only 7 of 16 plants were positively infested by *C. cinctus.* We observed a significant reduction of photosynthetic capacity of wheat plants as they senesced (Photo: $F = 76.9$, df = 4,29, $P < 0.0001$; g_s: $F = 4.52$, df = 4,29, $P = 0.0058$; C_i: $F = 6.88$, df = 4,29, $P = 0.0005$; E: $F = 12.1$, df = 4,29, $P < 0.0001$). No significant interactions were observed between time and treatment (uninfested and infested). A significantly lower net photosynthetic rate was observed for infested plants at each measuring event on days 17, 24, 31, 38, and 45 (Fig. 1A). The linear portion of the A-C_i response curve did not show any significant difference in CO_2 assimilation. However, a significant reduction in CO_2 assimilation was observed at the saturation point in infested plants (Fig. 2). No significant difference was observed in the light-response curve at any point of the curve (Fig. 3).

Study 2. We measured ≈60 flag leaves weekly during the period of 27 May to 20 June and 30 May to 23 June 2003 and 42 flag leaves during the period of 22 August to 3 September 2004. Because we observed a significant difference in the variance among runs, we analyzed the data separately. We did not observe significant differences for any photosynthetic parameter between infested and uninfested plants measured

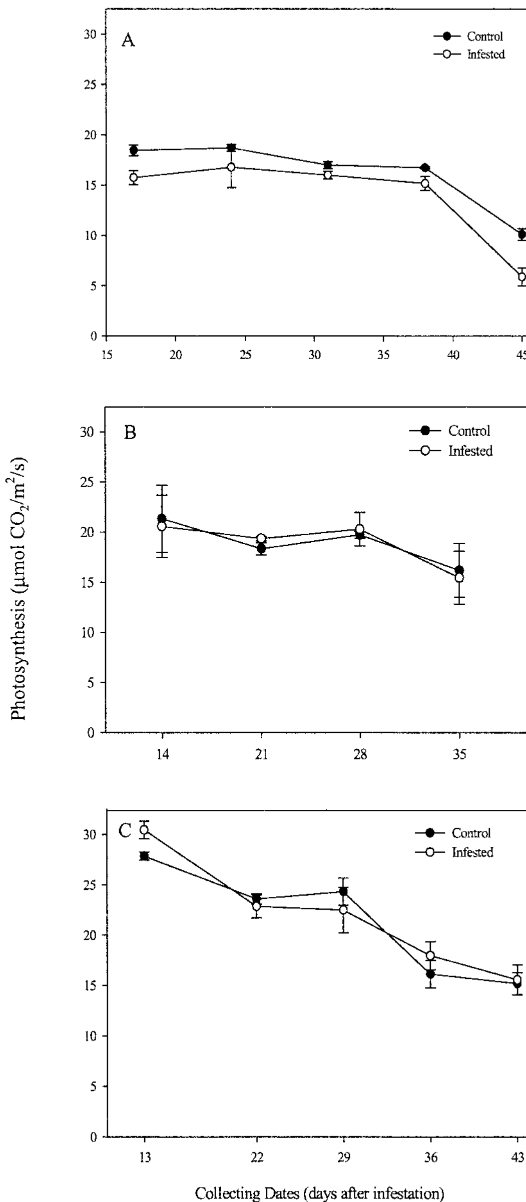

Fig. 1. Impact of *C. cinctus* on wheat photosynthetic rates measured over time (means ± SEM). (A) Wheat photosynthetic rates under environmental growth chamber measured at 17, 25, 31, 38, and 45 d after *C. cinctus* infestation. (B) Wheat photosynthetic rates under greenhouse environment measured at 14, 21, 28, and 35 d after *C. cinctus* infestation. (C) Wheat photosynthetic rates under field environment measured at 13, 22, 29, 36, and 43 d after *C. cinctus* infestation.

in all three greenhouse experiments. Conversely, significant reductions in photosynthetic capacity were observed as plants senesced in both 2003 experiments (experiment 1—Photo: F = 167.09, df = 3,21, P < 0.0001; g_s: F = 107.07, df = 3,21, P < 0.0001; C_i: F = 24.97, df = 3,21, P < 0.0001; E: F = 52.27, df = 3,21, P < 0.0001; experiment 2—Photo: F = 3.36, df = 3,21, P =

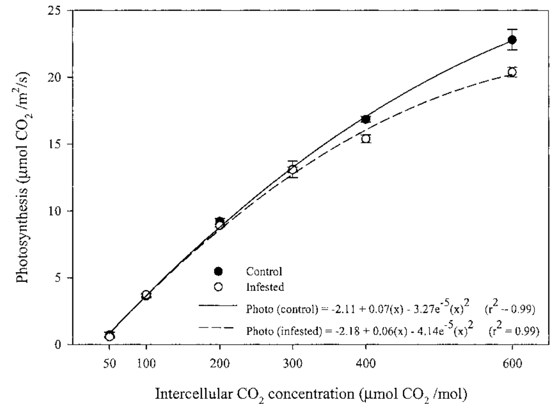

Fig. 2. A-Ci response curves to *C. cinctus* injury (means ± SEM).

0.0107; g_s: F = 84.85, df = 3,21, P < 0.0001; C_i: F = 20.61, df = 3,21, P < 0.0001; E: F = 11.86, df = 3,21, P < 0.0001). No significant interactions between time and treatment (infested and uninfested) were observed. Results from A-C_i and light-response curves were similar to those observed in study 1 (Fig. 2). Similar results were obtained from experiment 3 (2004). Briefly, *C. cinctus* did not elicit significant changes in any of the photosynthetic parameters measured, such as Photo, g_s, C_i, and E. We observed that photosynthetic values were lower than those observed in the two 2003 studies, which could be attributed to the more advanced developmental stage of the plants when they were first infested. However, the general pattern was consistent with previous greenhouse studies in which a significant reduction in these parameters was observed as plants senesced.

Study 3. We destructively sampled ≈225 plants within a period of three sampling events (i.e., 75 plants/event). A total of 304 stems were evaluated, and ≈60% were infested. We first observed eggs in the experimental plots on 17 June, and 67% of the eggs were located in tillers.

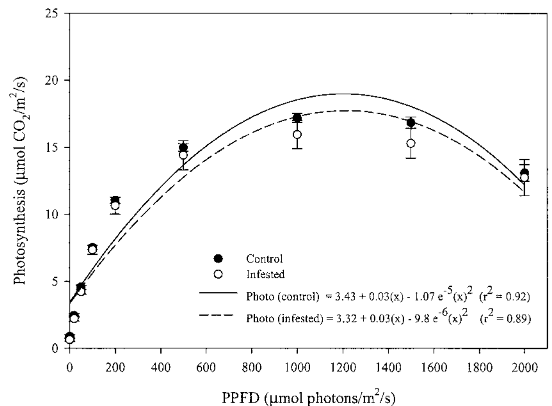

Fig. 3. Light response curve to *C. cinctus* injury (means ± SEM).

Table 1. Study 3 summary of mean ± SEM values for photosynthetic capacity (μmol CO_2/m/s) measured at days 13, 22, 29, 36, and 43 after *C. cinctus* infestation

	Days after infestation				
	13	22	29	36	43
Main Stem					
Control	30.8 ± 1.2a	26.4 ± 1.7b	23.8 ± 1.9b	18.8 ± 3.7c	15.6 ± 1.5c
Infested	31.7 ± 1.6a	24.2 ± 1.4b	25.1 ± 2.3b	17.0 ± 2.4c	17.8 ± 1.8c
Secondary stem					
Control	26.1 ± 0.2a	20.8 ± 1.5b	24.0 ± 2.7c	16.1 ± 1.6d	15.1 ± 1.6d
Infested	28.2 ± 1.7a	20.6 ± 1.5b	21.0 ± 3.0c	17.6 ± 1.9d	11.2 ± 1.6d

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

Primary metabolism measurements were first taken after we observed significant *C. cinctus* feeding on the parenchyma tissue. Frass resulting from larval feeding was observed on 30 June, 6 d after the first larva was found. Severe node injury (at least two of the three nodes damaged) was observed 10 d after we began taking physiological measurements. We did not observe significant effects of *C. cinctus* on the overall photosynthetic capacity measured in the period of the study (Photo: $F = 0.18$, df = 1,38, $P = 0.6749$; g_s: $F = 0.18$, df = 1,38, $P = 0.6696$; C_i: $F = 0.03$; df = 1,38, $P = 0.8585$; E: $F = 0.30$, df = 1,38, $P = 0.8780$). Conversely, we observed a significant reduction in wheat's photosynthetic capacity as plants senesced (Photo: $F = 50.72$, df = 4,38, $P < 0.0001$; g_s: $F = 58.01$, df = 4,38, $P < 0.0001$; C_i: $F = 5.75$; df = 4,38, $P = 0.001$; E: $F = 53.15$, df = 4,38, $P < 0.0001$; Fig. 1C). We observed a reduction on the photosynthetic capacity of ≈50% from growth stage 49–87 (Zadoks et al. 1974). We also observed a significant difference between main stem and tiller photosynthetic capacity ($F = 50.72$, df = 1,38, $P = 0.0001$). In general, tillers had a 14% lower photosynthetic capacity. Tillers also showed a reduced photosynthetic capacity as the plant approached growth stage 87 ($F = 2.90$, df = 4,38, $P = 0.0344$). A summary of wheat photosynthetic capacity results is presented in Table 1.

Cephus cinctus did not elicit significant impairment of chlorophyll *a* fluorescence on wheat flag leaves. None of the parameters measured with the fluorescence kinetic test was significantly affected by insect feeding (F_o: $F = 5.41$, df = 1,5, $P = 0.0676$; F_m: $F = 2.35$, df = 1,5, $P = 0.1861$; F_s: $F = 1.24$; df = 1,5, $P = 0.3277$; F_v/F_m: $F = 1.11$, df = 1,5, $P = 0.3406$; F_o': $F = 2.17$, df = 1,5, $P = 0.2003$; F_m': $F = 2.19$, df = 1,5, $P = 0.1991$; F_v'/F_m': $F = 1.28$, df = 1,5, $P = 0.3214$; qP: $F = 0.91$, df = 1,5, $P = 0.3400$; qN: $F = 1.11$, df = 1,5, $P = 0.3412$; NPQ: $F = 0.00$, df = 1,5, $P = 0.9964$; ETR: $F = 0.21$, df = 1,5, $P = 0.6672$). We also did not observe significant impact of tissue maturation on any of the fluorescence parameters.

Discussion

Despite the lack of significant effects of injury on wheat primary metabolic processes, with the exception of study 1 (i.e., growth chamber study), the responses observed in these studies are informative as we characterize how wheat responds physiologically to *C. cinctus* injury. Additionally, the responses are revealing as we consider if there are generalized physiological response modalities for the stem-boring injury guild.

Our results were inconsistent across different environments. Wheat leaves did not respond in a similar manner among different environments, suggesting that environmental conditions are important determinants for plant response to insect injuries. Wheat plants injured by *C. cinctus* under environmental growth chamber conditions clearly had their photosynthetic capacity impaired (Fig. 1A). Conversely, no significant reductions in photosynthetic capacity were observed in either greenhouse or field environments (Fig. 1B and C). These results support the hypothesis of Pedigo et al. (1986), which stated that environmental effects represent one of five key factors that determine how plants respond to arthropod herbivory.

The environmental conditions inside the environmental growth chamber used in this study were characterized by low-quality (intensity) light (≈63 μmol photons/m/s), concurrent with elevated atmospheric CO_2 concentrations (≈467 μmol CO_2/mol). Both of these abiotic factors are well documented to influence the overall photosynthetic capacity (Perchorowicz et al. 1981, Paul and Foyer 2001, Bidart-Bouzat et al. 2004, Melkonian et al. 2004, van Dongen et al. 2004, Van Heerden et al. 2004). Although light drives photosynthesis, it can also cause severe damage to the photosynthetic apparatus (Baroli and Melis 1998).

Recent studies by Johnson and Barber (2003) showed that low-light irradiance can have a similar impact on photosynthetic quantum yield. Light also seems to play an important role on the biochemical regulation of CO_2 fixation. Perchorowicz et al. (1981) showed that activation of $RuBP_2$ carboxylase (3-phospho-D-glycerate carboxylase [dimerizing]) is dependent on light intensity. They observed higher photosynthesis rates in leaves under higher irradiances, concurrent with increased inactivation of $RuBP_2$ carboxylase. In addition, light serves to coordinate assimilation of inorganic nitrogen with available carbon backbones produced during photosynthesis. Light also can affect plant gene expression by acting through phytochrome activation or through changes in levels of carbon metabolites (Oliveira et al. 2001).

CO_2 seems to be the most important limiting factor for the photosynthetic capacity of plants. Bryant et al. (1998) showed that plants exposed to elevated CO_2

concentrations for periods during their development showed significant reductions in gas exchange. Indeed, short-term photosynthetic responses of C_3 plants species to elevated CO_2 are usually modified by some degree of longer-term acclimation of the photosynthetic apparatus (Oechel and Strain 1995). For example, increases in global CO_2 concentration may alter plant compensation through increased biomass accumulation and photosynthetic rates (Bazzaz 1990, Hughes and Bazzaz 1997). However, it is important to point out that potential interactions with both abiotic and biotic limiting factors may result in over expression of damage by elevated CO_2 concentrations.

Considering the limiting factors discussed above and the results observed in this study, we suggest that plants under abiotically stressful environments, such as growth chamber environments, have their metabolic homeostasis altered. In such conditions, wheat plants are not able to tolerate *C. cinctus* injury. Indeed, a significant reduction in CO_2 assimilation was observed in the A-C_i response curve at the CO_2 saturation point, showing a limitation on assimilation may be caused by $RuBP_2$ carboxylase/oxygenase (Rubisco) activity inhibition. We compared the photosynthetic capacity of uninjured plants across the tested environments and found that plants under environmental chamber exhibited ≈24% lower gas exchange in relation to the rates observed in the greenhouse and field environments. Conversely, plants in better environmental conditions, such as in a greenhouse environment, seem to be able to tolerate *C. cinctus* injury. In our greenhouse environments, light quality and CO_2 levels were relatively better than in the growth chambers (≈334 μmol photons/m^2/s and 382 μmol CO_2/mol, respectively). Additionally, other potential limiting factors such as, nutrients, water, and temperature were better for wheat primary metabolism. The field environment during this study also was better, with ideal light quality, atmospheric CO_2 levels (376 μmol CO_2/mol), nutrients, and temperatures for wheat development.

Even though the primary metabolism parameters measured in this study are very sensitive to changes induced by plant stressors, we observed statistically significant gas-exchange reductions associated with *C. cinctus* injury only in the growth chamber study. Injury to wheat by *C. cinctus* is characterized by its subtlety. The injury is visually asymptomatic even though the larva often feeds inside the length of the entire stem, including the nodes. A more sensitive measure of effects on primary physiology is chlorophyll fluorescence. However, we did not observe any differences in fluorescence. Therefore, the injury seems to be not only visually, but also physiologically, asymptomatic under certain environmental conditions.

Injury by *C. cinctus* has been detected using other physiological parameters. Studies evaluating plant volatiles produced by infested and uninfested wheat plants in the greenhouse show relatively subtle changes in the amounts of certain compounds produced. In general, infested plants produce more volatiles than uninfested; however, a few volatile compounds seem to be less abundant for infested (D.K.W. and W.L.M., unpublished data). Elevated levels of ripening compounds appear for infested plants 1–2 wk earlier than for control plants, suggesting premature senescence (D.K.W. and W.L.M., unpublished data). Another series of greenhouse experiments evaluating varietal variation in wheat volatile production show that nutrient limitation and reduced light intensity both decrease volatile production (D.K.W. and W.L.M., unpublished data).

In addition to yield loss because of lodging and breakage of stems, wheat stem sawfly injury also results in physiological yield loss (Morrill et al. 1992a). Physiological yield loss, of course, implies impairment of physiological processes. However, based on this study, more research is needed before the physiological mechanism or group of mechanisms underlying yield loss are more thoroughly understood.

Pedigo et al. (1986) suggested that responses of plants to arthropod injury are dependent on five factors: (1) time of injury, (2) plant part injured, (3) injury type, (4) intensity of injury, and (5) environmental effects. Results from this study indicate that physiological responses of wheat to *C. cinctus* stem-boring injury may be highly dependent on environmental interactions. This is not unexpected. Indeed, the ability of abiotic stressors to interact with biotic stressors is well known (Haile 2001, Peterson and Higley 2001).

The interactions among arthropod injury, plant responses, and environmental factors necessitates that characterizations of physiological responses also contain detailed descriptions of abiotic conditions. This is salient not only for characterizing a single host plant and herbivore species, but also for developing generalized models of response to an injury guild.

This idea of injury guilds based on injury development (i.e., symptoms) to physiological response has been purposed for defoliators. Higley et al. (1993) divided the defoliation injury type into the following three injury guilds: leaf-mass reduction, leaf photosynthetic rate reduction, and leaf senescence alteration. Based on our findings in this study and previous studies, it seems likely that there is no generalized model of plant physiological response to stem boring. This is not surprising because "stem boring" currently refers to a type of injury based on physical appearance. Therefore, it seems likely that the stem-boring injury guild also will need to be divided into additional guilds based on physiological response.

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