

Photosynthesis and Yield Reductions From Wheat Stem Sawfly (Hymenoptera: Cephidae): Interactions With Wheat Solidness, Water Stress, and Phosphorus Deficiency

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ABSTRACT The impact of herbivory on plants is variable and influenced by several factors. The current study examined causes of variation in the impact of larval stem mining by the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), on spring wheat, *Triticum aestivum* L. We performed greenhouse experiments over 2 yr to 1) study whether biotic (hollow versus solid stemmed host wheat) and abiotic (water, phosphorus stress) factors interact with *C. cinctus* stem mining to influence degree of mined stem physiological (photosynthesis) and yield (grain weight) reductions; and 2) determine whether whole plant yield compensatory responses occur to offset stem-mining reductions. Flag leaf photosynthetic reduction was not detected 16–20 d after infestation, but were detected at 40–42 d and doubled from water or phosphorus stresses. Main stem grain weight decreased from 10 to 25% from stem mining, largely due to reductions in grain size, with greater reductions under low phosphorus and/or water levels. Phosphorus-deficient plants without water stress were most susceptible to *C. cinctus*, more than doubling the grain weight reduction due to larval feeding relative to other water and phosphorus treatments. Two solid stemmed varieties with stem mining had less grain weight loss than a hollow stemmed variety, so greater internal mechanical resistance may reduce larval stem mining and plant yield reductions. Our results emphasize the importance of sufficient water and macronutrients for plants grown in regions impacted by *C. cinctus*. Also, solid stemmed varieties not only reduce wheat lodging from *C. cinctus*, they may reduce harvested grain losses from infested stems.

KEY WORDS herbivory, stem mining, phosphorus deficiency, water stress, biotic

The impact of herbivory on plants can be highly variable (Machinski and Whitham 1989, Paige 1999, Delaney and Macedo 2001). Such variation in damage from herbivory injury can result from multiple factors such as type of injury (Pedigo et al. 1986, Delaney and Macedo 2001, Peterson 2001), environmental variation (Maschinski and Whitham 1989, Haile and Higley 2003), genetic variation (Haile et al. 1999, Paige 1999), degree of investment into reproduction (Chiariello and Gulman 1991) or defense (Herms and Mattson 1992), and timing of injury (Knight 2007). Several plant compensatory response mechanisms occur after herbivory that can influence how herbivory translates into plant damage (Trumble et al. 1993).

Because environmental variation can be important in affecting plant response to herbivory, two limiting plant resources that have been manipulated in herbivory response studies are nitrogen and water (Coughenour et al. 1985, Maschinski and Whitham 1989, Sadras et al. 1998). Another resource known to be limiting to plant growth and yield is phosphorus

(Plénet et al. 2000) and to compensatory regrowth after herbivory (Chapin and McNaughton 1989). In Montana, soils tend to have low phosphorus levels (Fixen 2001, Jones and Jacobson 2005) and only 20–30 cm of precipitation each year (Parrett 1997). A crop such as spring wheat, *Triticum aestivum* L., frequently grows under stressful dryland conditions with low soil phosphorus.

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is an economically important stem-mining herbivore of Northern Great Plains wheat (Wallace and McNeal 1966, Morrill et al. 2000, Beres et al. 2007). One or more eggs are deposited into a wheat stem in the field in late May through early July depending on local conditions, but because of cannibalism only one larva will survive within a stem. Under heavy *C. cinctus* infestation, as many as 95% stems in a wheat field can lodge, making harvest more expensive and time-consuming, and uncollected heads further reduce yield. The second form of wheat yield loss from *C. cinctus* stem-mining herbivory is reduced head mass on infested stems that are harvested (Holmes 1977), especially from injury to vascular tissues.

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Highly variable head mass reductions from 2 to 30% have been reported from *C. cinctus* stem mining (Seamans et al. 1944, McNeal et al. 1955, Wallace and McNeal 1966, Holmes 1977, Morrill et al. 1992). Part of the difficulty in measuring head mass reduction is that *C. cinctus* females have a known preference to oviposit on larger diameter stems, which tend to have larger heads (Morrill et al. 2000, Buteler et al. 2008 with Corrigendum 2009). One approach to address this problem has been to collect infested and uninfested wheat stems from the field to examine the degree to which head mass is below the head's reproductive potential (Buteler et al. 2008 with Corrigendum 2009). A different approach is to match the size of control and infested treatment plants at the time of infestation in a greenhouse (Macedo et al. 2007). Matched plants should allow for similar reproductive potentials between control and infested treatment plants so that we can study the impact of *C. cinctus* herbivory on spring wheat heads.

Our research examines biotic and abiotic stress interactions that affect a wheat plant's primary physiology and subsequent yield. We seek to determine the impact of *C. cinctus* on main stem yield by using one hollow stem and two solid stemmed spring wheat varieties that differ in *C. cinctus* lodging resistance, and examine whether water and/or phosphorus deficiency influence the magnitude of yield reduction due to *C. cinctus*. We conducted 2 yr of our experiment in a greenhouse, where we matched main stem height of infestation and control treatment plants at the beginning of *C. cinctus* adult female introduction. Main stem flag leaf photosynthetic rate was measured twice after infestation (once during anthesis and a second time during grain filling/early plant senescence) to detect plant stress in growing plants. Main stem yield parameters (grain mass, grain number, and individual grain size) were measured to examine the impact of stem-mining herbivory, whereas yield parameters from uninfested tillers were measured to test for whole plant yield compensatory responses. Other plant growth parameters (main stem height and stem solidness; whole plant dry mass and tiller number) were measured to determine if sawfly herbivory also affected components of nonreproductive plant growth.

Materials and Methods

Greenhouse Conditions. Experiments were conducted in 2007 and 2008 in a greenhouse at the Montana State University Plant Growth Center. Plants were grown in square 10.2-cm pots in a 1:1 mix of a sunshine soil medium and sand, and they were maintained for the duration of the study at $23 \pm 3^\circ\text{C}$, 20–40% RH, and a photoperiod of 14:10 (L:D) h. Supplemental GE Multivapor Lamps (MVR 1000/C/U, GE Lighting, General Electric Company, Cleveland, OH) were used to enhance light intensity in the greenhouse during the morning and evening.

Experimental Factors. The experiment had a 4 by 3 by 2 by 2 randomized complete block experimental design involving four water \times phosphorus treatments

(well watered with phosphorus supplementation [W+P+], well watered without phosphorus supplementation [W+P], low water with phosphorus supplementation [W-P+], and low water without phosphorus supplementation [W-P-]), three spring wheat varieties ('Reeder', 'Ernest', and 'Choteau'), two wheat stem sawfly exposure treatments (control and exposed), and 2 yr in which the experiment was conducted (2007 and 2008). There were three different outcomes from main stem exposure to *C. cinctus* adults resulting in four total sawfly exposure treatments: 1) control uninfested—stems that received a tube but no exposure to *C. cinctus* adults, 2) sawfly uninfested—stems that received a tube and sawfly adult exposure that subsequently showed no signs of larval infestation or herbivory (frass absent) during stem splitting thus had no stem cutting, 3) infested uncut—stems that received a tube and sawfly adult exposure that subsequently showed signs of larval infestation and herbivory (frass present) during stem splitting but were not cut because of larval death, and 4) infested cut—stems that received a tube and adult sawfly exposure which were subsequently cut and therefore had larval infestation and herbivory in the stem (frass present). In the 2007 experimental run, there were 14 complete replicates of 24 plants per replicate, so 336 plants were included. In the 2008 experimental run, there were nine complete replicates of 24 plants/replicate and a few additional infested plants from poorly infested replicates, so a total of 228 plants were included. Plants were rotated along their bench weekly to randomize greenhouse variation in sunlight, temperature, and air flow.

Of the three spring wheat varieties included, Reeder and Choteau typically have greater yields than Ernest. The varieties also differ in stem solidness in a scale where 5 is the minimum (fully hollow stem with all five internodes) and 25 the maximum (stem filled with pith in all five internodes) level of solidness (Larson 1959). Higher stem solidness helps to reduce stem lodging from *C. cinctus*. Reeder is a hollow stem variety (scores of 5–7), Ernest has moderate stem solidness (scores of 12–17), and Choteau is the most consistently solid stemmed spring wheat variety (scores of 19–24) under sufficient light conditions. We tested whether Ernest and Choteau would be less sensitive to the impact of *C. cinctus* herbivory on reducing a wheat stem's grain mass than hollow-stemmed Reeder. Three seeds of a spring wheat variety were weighed with a microscale precise to 0.1 mg (Explorer model, Ohaus, Pine Brook, NJ) and then seeded into a pot. Pots with two or three seedlings were used in our experiments.

The phosphorus treatment was imposed when wheat seedlings reached Zadoks 13 (three-leaf stage; Zadoks et al. 1974). At that stage, 250 ml of fertilizer was provided to each pot twice each week. The phosphorus-deficient fertilizer (P-) was prepared from a N:P:K mix with micronutrients (Peters 15-0-15, Scotts-Sierra Hort. Prod. Company, Maryville, OH) to provide 100 ppm fertilizer that only lacked phosphorus. For phosphorus-supplemented fertilizer (P+),

the same amount of 15-0-15 N:P:K mix was used with the addition of phosphorus pentoxide (P_2O_5 ; Sigma-Aldrich, St. Louis, MO) to provide 100 ppm fertilizer. Pots were watered as needed before the water and infestation treatments started.

Because *C. cinctus* requires an internode for oviposition, the herbivory treatment could not begin until the stem had two nodes or reached 32 on the Zadoks scale (Zadoks et al. 1974). Depending on adult *C. cinctus* availability, infestation began between Zadoks 32 and Zadoks 49 (end of booting), although primarily from Zadoks 32 to Zadoks 39 (emergence of the flag leaf). One or two replicates were infested on a given day. Plexiglas infestation tubes had a 4-cm diameter and 60-cm height. A tube was placed over the main stem of one plant on a pot with other tillers excluded. Stem heights were matched between control and herbivory pots within each variety for each water or phosphorus treatment. Each herbivory treatment pot received three adult *C. cinctus* females and one male per tube over one plant in a pot, whereas a control stem within a tube received no sawflies. The sawflies were held in the tube over the main stem for 3 d. We chose main stem herbivory because the main stem is easily distinguished as a plant's tallest stem during elongation and booting (Zadoks 31-49) and because a plant invests primarily in maintaining its main stem growth under limited soil moisture conditions (Cabeza et al. 1992; unpublished data).

After infestation ended, the water treatment began. For well watered treatment pots, a relative soil moisture level of 800-1000ADC was maintained using soil moisture sensor readings (Echo Check 5, Decagon Devices Inc., Pullman, WA), whereas low water treatment pots had a relative soil moisture maintained at <600ADC but kept above wheat's permanent wilting point. Three times a week, soil moisture readings were collected from all pots. Well-watered pots received 2 ml of water/ADC < 850, whereas low water pots received 1 ml of water/ADC < 600. Well watered pots were also watered on the other 4 d, so the 3 d of weekly soil moisture checks allowed for soil moisture adjustments with individual pots. Low water treatment pots did not receive extra water on the other 4 d unless early signs of wilting were detected. After watering each day, each pot received 70 ml of their P+ or P- fertilizer so that at a minimum, plants received daily moisture to allow for chronic water stress in the low water treatment pots.

Gas Exchange Measurements. An infrared gas analyzer (model LI-6400-40; Li-Cor Biosciences, Lincoln, NE) with a simultaneous gas exchange/chlorophyll a fluorescence chamber (2 cm²) was used to collect photosynthesis measurements from main stem flag leaves. A light intensity of 1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was set with an internal red/blue light source inside the measurement chamber, a mixer set reference CO₂ to 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to reach the chamber, and air flow was set at 250 $\mu\text{mol s}^{-1}$. Measurements were collected from 1130 to 1600 hours at 20 d (anthesis: Zadoks 61-70) and 40 d (seed dough filling; Zadoks 81-90) after sawfly introduction in 2007; also

at 16 d (early anthesis: Zadoks 61-65), 30 d (Zadoks: 71-79: seed milk filling), and 42 d (Zadoks 81-92: dough formation and early senescence) in 2008. Due to similar trends, data from 20 d in 2007 and 16 d in 2008 were combined for analysis, and data from 40 d in 2007 and 42 d in 2008 were combined for analysis. For brevity, we only report on photosynthetic rate from gas exchange and chlorophyll a fluorescence data.

Plant Growth and Yield Measurements. After plants senesced, growth and yield data were collected. Main stem height was measured from all control and *C. cinctus* exposed plants. Each infestation treatment main stem head was cut off and weighed (Scout II Scale, Ohaus), threshed (Precision Machine, Lincoln, NE), and grains were weighed and counted; the same process was repeated for pooled remaining tiller heads of treatment plants. After head processing, nonreproductive shoot dry mass was weighed. Stem solidness was then determined by cutting the middle of each internode and providing a score from 1 (hollow, no pith) to 5 (solid with pith) following Larson (1959). The internode scores were summed to characterize the overall stem solidness score. All lodging had occurred by this point, so (infested) cut plants were readily visible. The main stem of uncut treatment plants was split to check for the presence of *C. cinctus* frass to determine stems where stem mining occurred where the larva died (infested, uncut) versus stems lacking frass to indicate a lack of larval feeding (uninfested).

Statistical Analysis. A univariate mixed model analysis of covariance (ANCOVA) was analyzed using Proc GLM with SAS version 9.2 (SAS Institute 2007) for each dependent variable. The analyses included block and year as random factors, initial seed mass was a continuous covariate, number of plants in the pot (two or three) to account for plant density as a discrete covariate, and crossed fixed factors (variety, water \times phosphorus, and infestation). Initial seed mass refers to the mass of the seed used to generate an experimental plant, and has been positively correlated to plant yield in some studies (D.K.W., unpublished data). Thus, we treated initial seed mass as a covariate to account for any whole yield or growth parameters that might be affected by the size of the seed that germinated experimental plants. Head weight and grain weight were highly correlated and had the same trends in the results, so we report on grain weight, grain number, and average grain size (individual grain weights) for main stem yield components, as well as from the other tillers. For photosynthetic rate data, we used repeated measures GLM and then univariate GLM for each postinfestation measurement time (20 d, 40 d) with SAS 9.2 (SAS Institute 2007). Fisher protected least significant difference (LSD) post hoc test was used to detect treatment differences when infestation was significant ($\alpha = 0.05$), because we were trying to detect treatment differences when they occurred and excessive comparisons were not involved.

Table 1. Univariate GLM analyses of variance of spring wheat flag leaf photosynthetic rate at anthesis (Pnant) and early senescence (PnES); main stem grain mass (GMM), number (G#M) and main stem average individual grain mass (GAM); other tiller grain mass (GMO), number (G#O), and other tiller average individual grain mass (GAO); height of main stem (HtM); stem and leaf whole plant dry mass (SLMass); total plant tillers and main stem (T); and main stem solidness (SolM)

Factor	df	Pnant	PnES	GMM	G#M	GAM	GMO	G#O	GAO	HtM	SLMass	T	SolM
Yr	1,477	68***	20***	18***	ns	31***	162***	121***	28***	54***	130***	177***	11***
Block (yr)	23,477	10***	3.9***	3.8***	5.6***	4.1***	4.1***	4.3***	3.8***	11***	10***	3.0***	2.7***
Germinal seed mass	1,477	ns	6.3*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Plants/pot	1,477	ns	ns	ns	4.7*	ns	97***	107***	ns	13***	145***	80***	ns
Infestation (I)	3,477	ns	3.3*	15***	ns	18***	ns	ns	2.6*	3.7**	ns	ns	3.5*
Variety (V)	2,477	ns	ns	6.5**	8.8***	ns	6.2**	5.3**	ns	173***	8.4***	8.2***	936***
Water/phosphorus (WP)	3,477	125***	34***	8.8***	3.2*	13***	152***	144***	7.9***	154***	111***	133***	4.1**
V × I	6,477	ns	ns	ns	ns	3.6**	ns	ns	ns	ns	ns	ns	ns
WP × I	9,477	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
V × WP	6,477	ns	2.3*	ns	ns	ns	2.2*	2.1*	ns	ns	ns	2.4*	ns
V × WP × I	18,477	ns	ns	ns	ns	ns	2.1**	2.0**	ns	ns	2.0**	2.6***	ns

ns, $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Results

In 2007, 50% of stems were stem-mined as 47 stems were cut, 41 stems were mined but not cut (infested), and 88 stems were uninfested; there were also 167 control stems. In 2008, 26.5% of stems had stem mining (20 stems were cut, 10 stems were mined but not cut, and 81 stems were uninfested; there were also 111 control stems). At 16–20 d after infestation (plants in anthesis), water stress was the only factor to significantly reduce main stem flag leaf photosynthetic rate (Table 1) by 30–50% (Fig. 1A and B).

Main Stem Leaf Photosynthesis. Phosphorus deficiency, *C. cinctus* infestation, and water stress affected photosynthetic rate of early senescing plants 40–42 d after infestation based on significant repeated measures ANCOVA date × infestation ($F_{3,510} = 2.88$; $P = 0.04$), date × water/phosphorus ($F_{3,510} = 11.3$; $P < 0.001$), and date × infestation × water/phosphorus ($F_{9,510} = 1.91$; $P = 0.05$) terms (also see Table 1). Flag leaf photosynthesis on stem-mined and subsequently cut main stems was significantly lower than on leaves from other treatments (controls, unmined *C. cinctus* exposed plants, and stem-mined but uncut plants; Table 2; Fig. 1A). Reeder’s photosynthetic reductions of 40% were almost double the reductions for Ernest and Choteau (Fig. 1A). Flag leaf photosynthetic rate on mined (but uncut) plants was not significantly different from rates on unmined plants (Table 1), although there were trends of 15–20% reductions for Reeder and Ernest, and Choteau had a trend for a 25% increase (Fig. 1A). The magnitude of infestation effects interacted with the water/phosphorus conditions the plant experienced (Fig. 1B). Flag leaf photosynthetic reductions were nearly doubled in mined stems on plants having water or phosphorus stress compared with unmined stems, but small or no reductions occurred in mined stems on either unstressed plants or plants with both water and phosphorus stresses (Fig. 1B).

Main Stem Yield. Water stress, phosphorus stress, and *C. cinctus* stem mining reduce grain weight (Tables 1 and 2; Fig. 2). Stem mining caused significant

grain weight reductions compared with control and sawfly exposed unmined stems, with a nonsignificant trend for smaller reductions in mined but uncut stems compared with mined and cut stems (Table 2). Grain weight from solid stemmed varieties Ernest and Choteau had smaller reductions than hollow stemmed Reeder (Fig. 2A). A 10–15% grain reduction occurred in plants with water and/or phosphorus stress (Fig.

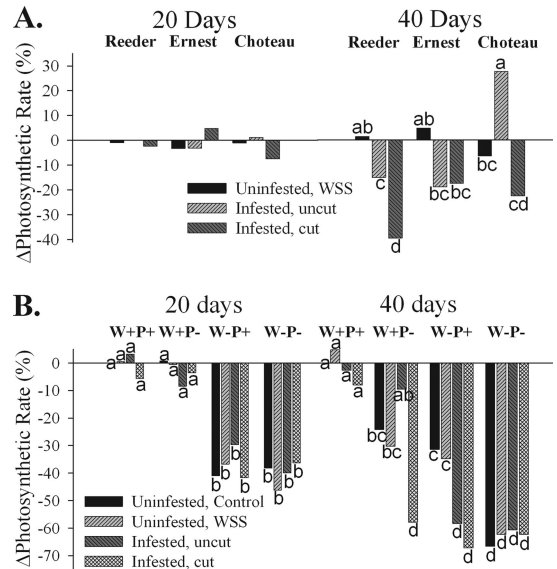


Fig. 1. Relative percentage difference [(treatment_{mean} – control_{mean})/control_{mean}] for main stem flag leaf photosynthetic rate is shown for wheat stem sawfly treatments fully crossed with spring wheat varieties (A) or water × phosphorus treatments (B) at 16–20 d and also 40–42 d after infestation. For each spring wheat variety in A, three infestation outcomes (uninfested, infested but uncut, infested, and cut) are compared with control treatment stems (controls are set to 0% difference). For the water × phosphorus treatments in B, the unstressed control plants are set to 0% difference and are compared with all 15 other infestation × water/phosphorus treatment means.

Table 2. Mean \pm SE spring wheat flag leaf photosynthetic rate at anthesis (PnES); main stem grain mass (GMM), number (G#M) and main stem average individual grain mass (GAM); other tiller grain mass (GMO), number (G#O), and other tiller average individual grain mass (GMO); stem and leaf whole plant dry mass (SLMass); total plant tillers and main stem (T); and main stem solidness (SolM)

Treatment	Sample	Pnant	PnES	GMM	G#M	GAM	GMO	G#O	GAO	HtM	SLMass	T	SolM
Control, unfested	n = 278	18.3 \pm 0.28	8.3 \pm 0.37A	1.29 \pm 0.019A	37.6 \pm 0.45	34.4 \pm 0.33A	2.19 \pm 0.07	69.5 \pm 2.0	31.3 \pm 0.42A	74.1 \pm 0.43A	3.32 \pm 0.06	3.54 \pm 0.07	13.5 \pm 0.58B
Sawfly, unfested	n = 169	18.0 \pm 0.34	8.3 \pm 0.45A	1.27 \pm 0.023A	37.5 \pm 0.56	34.1 \pm 0.42A	2.18 \pm 0.069	69.9 \pm 2.5	31.1 \pm 0.53A	73.2 \pm 0.54A	3.35 \pm 0.07	3.46 \pm 0.08	14.1 \pm 0.23AB
Infested, uncut	n = 51	18.2 \pm 0.62	8.1 \pm 0.81A	1.13 \pm 0.046B	37.0 \pm 1.14	31.0 \pm 0.84B	1.92 \pm 0.17	67.1 \pm 4.9	28.9 \pm 1.07B	73.1 \pm 1.07AB	3.07 \pm 0.15	3.43 \pm 0.17	14.0 \pm 0.45AB
Infested, cut	n = 67	18.0 \pm 0.54	6.0 \pm 0.71B	1.06 \pm 0.036B	35.7 \pm 0.87	30.0 \pm 0.65B	2.21 \pm 0.14	69.6 \pm 4.1	31.3 \pm 0.86A	71.2 \pm 0.83B	3.25 \pm 0.11	3.34 \pm 0.13	14.6 \pm 0.35A

For infestation factors with significant effects in Table 1, treatments followed by the same letter are not significantly different ($\alpha = 0.05$), Fisher protected LSD post hoc test. Units: Pnant and PnES, μ mol CO₂ m⁻² s⁻¹; GMM, GMO, and SLMass, g; GAM and GAO, mg; HtM, cm; and SolM, range of 5–25.

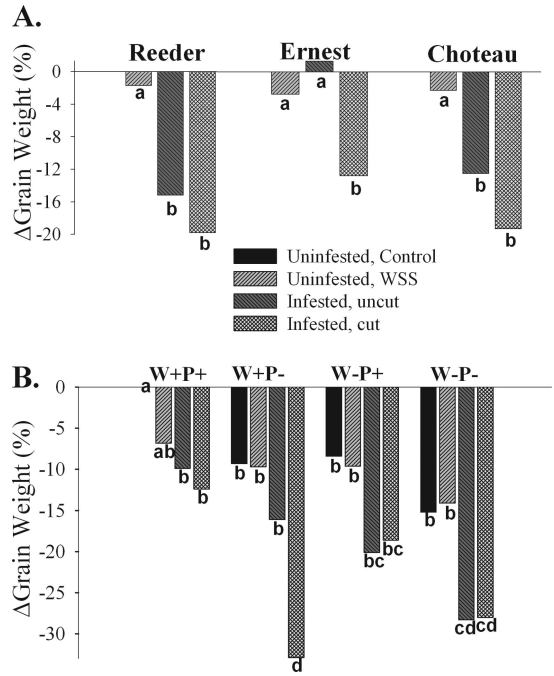


Fig. 2. The relative percentage difference [(treatment_{mean} - control_{mean})/control_{mean}] for main stem grain weight is shown for wheat stem sawfly treatments fully crossed with spring wheat varieties (A) or water \times phosphorus treatments (B). See Fig. 1 legend for details.

2B). Water and phosphorus stresses also influenced the degree of grain weight reductions from *C. cinctus*. Grain weight reductions from stem mining were an additional 10–17% for most water \times phosphorus treatments, but cut main stems of phosphorus deficient plants experienced a 25% reduction from *C. cinctus* to result in a total grain weight reduction of 35% (Fig. 2B).

Herbivory was not a significant term in the analysis for grain number (Table 1). There was a trend for small reductions of 4–8% in mined and cut stems across all varieties, but little or no reductions for mined stems that remained uncut (Table 2; Fig. 3A). Grain kernel number reductions of 0–10% occurred from stem mining under the water \times phosphorus treatments (Fig. 3B). In contrast, herbivory was a significant term for the analysis of grain kernel size (Table 1), and was significantly reduced for both cut and uncut mined stems (Table 2). A significant herbivory \times variety interaction (Table 1) reflected that Reeder plants had greater kernel size reductions of 20% due to mining, whereas Ernest and Choteau had kernel size reductions of 5–10% when mined (Fig. 4A). Main stem kernel size reductions from herbivory ranged from 5 to 20% under different water \times phosphorus conditions, with the largest reductions in cut stems on phosphorus stressed plants with adequate water (Fig. 4B).

Other Yield and Plant Growth Parameters. Although water \times phosphorus, variety, and plant density

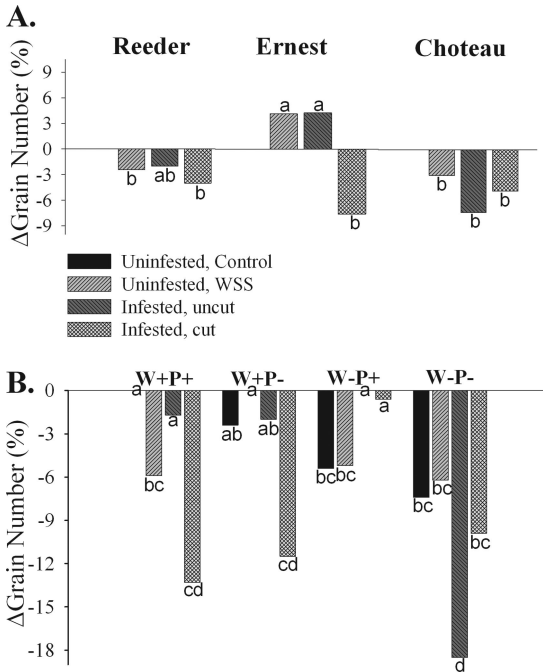


Fig. 3. Relative percentage difference $[(\text{treatment}_{\text{mean}} - \text{control}_{\text{mean}}) / \text{control}_{\text{mean}}]$ for main stem grain number is shown for wheat stem sawfly treatments fully crossed with spring wheat varieties (A) or water \times phosphorus treatments (B). See Fig. 1 legend for details.

tended to be important factors for grain yield on other plant tillers, main stem herbivory tended not to be an important factor in influencing yield of uninfested tillers or other whole plant parameters like shoot mass and tiller number (Tables 1 and 2). Mined main stems that were cut had slightly reduced (<5% reduction) height, and were slightly more solid (by a score of 1) than controls (Table 2).

Discussion

Stem Mining and Flag Leaf Photosynthesis. Larval *C. cinctus* stem mining affected main stem flag leaf photosynthetic activity and subsequent grain weight. Yet, main stem mining did not result in any detectable negative or positive (compensatory) whole plant responses with uninfested tillers. Photosynthetic rate reductions were not detected in the first set of measurements during anthesis 16–20 d after initial infestation. This finding is to be expected because insect larval growth tends to be exponential. As such, a larva at 16–20 d postinfestation will typically still be in an earlier developmental stadium, and so little of the overall stem mining will have accumulated. In early senescing plants 40–42 d after initial oviposition, significant flag leaf photosynthetic rate reductions were detected. By this time, a *C. cinctus* larva is probably in its last stadium and will consume the majority of plant tissue required for its development. Most stem feeding injury occurred in the basal one to two internodes,

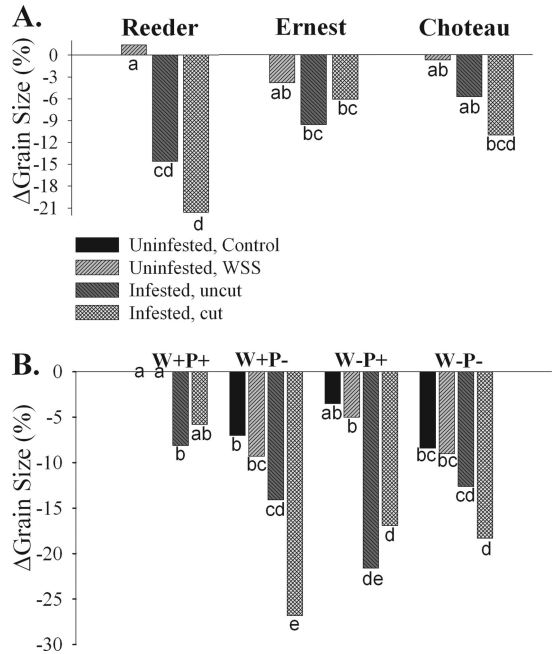


Fig. 4. Relative percentage difference $[(\text{treatment}_{\text{mean}} - \text{control}_{\text{mean}}) / \text{control}_{\text{mean}}]$ for main stem average grain size is shown for wheat stem sawfly treatments fully crossed with spring wheat varieties (A) or water \times phosphorus treatments (B). See Fig. 1 legend for details.

sufficient to result in significant mined stem yield reductions. In the field, stem mining can occur along all four or five stem internodes and injure all nodes (D.K.W., unpublished data); so, future greenhouse studies should provide estimates of stem yield reduction across a wider range of *C. cinctus* mined internodes and nodes. Stem-mining injures vascular tissue when tunneling through nodes, suggested to inhibit vascular transport of water and nutrients to developing grain (Morrill et al. 1992).

Other studies have detected flag leaf photosynthetic reductions in *C. cinctus* mined wheat stems in growth chamber and greenhouse conditions (Macedo et al. 2005, 2007). Macedo et al. (2007) detected photosynthetic reductions at 28 d postinfestation during grain filling. In 2008, we observed a trend of photosynthetic rate reductions at 32 d postoviposition, but the percentage of mined stems was too low for the decrease to be detected with statistical significance (data not shown). However, by 40–42 d postoviposition, photosynthetic rate reductions were statistically significant for the 2007 data along with a nonsignificant trend for the 2008 data (K.J.D., unpublished data).

Of the few studies examining leaf photosynthetic responses after stem boring or mining, photosynthetic rate decreases have been reported with corn, *Zea mays* L., and potato, *Solanum tuberosum* L. (Godfrey et al. 1991, Ziems et al. 2008). However, increases in leaf photosynthetic rates have been reported from leaves on insect bored rice stems, *Oryza sativa* L., so plant compensatory responses can occur even with vascular

disruption (Rubia et al. 1996). An even more unique finding was an inhibitory systemic effect from stem boring, where leaves from a *S. tuberosum* stem without injury often had photosynthetic reductions when stem boring injury occurred on a different stem of plant (Ziems et al. 2008). Also, *S. tuberosum* leaf photosynthetic impairment was suggested to be caused by mesophyll (reduced carboxylation) rather than stomatal limitation (Ziems et al. 2008), a conclusion we also draw for our results based on significant increases in leaf intercellular leaf CO_2 (C_i) in mined stems (data not shown).

Our experiments extend the findings of Macedo et al. (2007) by distinguishing whether mined main stems were ultimately uncut or cut. In doing so, we found that mined stems that were ultimately cut had photosynthetic reductions at 40–42 d postinfestation to support the results of Macedo et al. (2007). Yet, we also showed that mined stems where the *C. cinctus* larva died (stem was never cut) had no difference in photosynthetic rate relative to control and uninfested stem treatments. In fact, with the very solid Choteau stems that were infested but uncut, compensatory photosynthesis was statistically significant despite yield reductions.

Macedo et al. (2005) initially detected leaf photosynthetic reductions from *C. cinctus* herbivory only under low-light growth chamber conditions but not under greenhouse or field conditions. They suggested that the low-light conditions stressed the wheat sufficiently such that the additional stress of stem-mining herbivory further impaired leaf photosynthesis. Increased head photochemical efficiency and chlorophyll content was detected to help explain why head mass (and thus grain) reductions were not detected in mined stems in the greenhouse (Macedo et al. 2006, 2007). Our current results suggest that photosynthetic rate reductions may require sufficient *C. cinctus* stem-mining duration. Leaf stomatal conductance reductions combined with intercellular leaf [CO_2] increases suggest mesophyll limitations to photosynthesis reflected in reductions in chlorophyll fluorescence parameters such as maximal and effective photochemical efficiencies, and nonphotochemical quenching (data not shown). Future work needs to examine photosynthetic activity of all leaves along a mined wheat stem and to look for a relationship between degree of *C. cinctus* grain mass reductions with leaf photosynthetic reductions.

Wheat flag leaf photosynthetic reductions of 40–50% in our experiments resulted from low soil moisture and reductions of 10–20% from chronic phosphorus deficiency, similar results to previous studies (Macedo et al. 2006; T.M.M., unpublished data). The impact of *C. cinctus* herbivory on main stem flag leaf photosynthesis was also influenced by the water \times phosphorus conditions the wheat plant experienced, supporting the findings of previous research (T.M.M., unpublished data). Unstressed plants and plants with both water stress and no phosphorus supplementation had small photosynthetic rate reductions <10%. In contrast, plants with either phosphorus stress or water

stress alone had flag leaf photosynthetic reductions of 25–35% and these reductions were nearly doubled for stems that were additionally mined and cut. Thus, the least and most stressed plants had the smallest photosynthesis decreases from herbivory, whereas plants with one of the two stresses had much larger photosynthetic reductions. This finding is similar to a field study with mechanical tissue removal, plant competition, and water supplementation with common milkweed, *Asclepias syriaca* L. (Delaney 2003). Other studies have also shown that plants with severe drought stress in the field are more susceptible to photosynthetic reductions from European corn borer, *Ostrinia nubilalis* (Hübner), stem boring on cornstalks (Godfrey et al. 1991), and western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Dunn and Frommelt 1998), injury on corn roots; yet, well watered plant leaves were most susceptible to photosynthetic impairment from spider mite herbivory, *Tetranychus urticae* Koch, on soybean, *Glycine max* (L.) Merr., leaves (Haile and Higley 2003). More stressed plants may also be more sensitive to larger photosynthetic reductions from the additional stress of insect herbivory.

Main Stem Yield and Stem Mining. We also expanded on the results of Macedo et al. (2006, 2007) by showing that *C. cinctus* herbivory caused a grain weight reduction for infested stems, primarily by reducing individual grain kernel size. Matching main stem height for control and infestation treatments eliminated adult female oviposition preference for larger stems, which was confirmed as control and *C. cinctus* exposed uninfested stem treatments were similar for all measured photosynthetic, growth, and yield parameters. Mined stem grain weight reductions ranged from 10 to 25% compared with control, unstressed plants. Previous studies had reported quite variable reductions of 2–30% depending on the study (Seamans et al. 1944, McNeal et al. 1955, Wallace and McNeal 1966, Holmes 1977, Macedo et al. 2007). The variability in grain weight reduction we observed was explained more by differences in the environmental conditions the plants experienced via water stress and phosphorus deficiency than by genetic differences in wheat varieties. Grain weight reductions caused by *C. cinctus* were somewhat smaller in the solid stemmed Ernest and Choteau varieties than hollow stemmed Reeder. However, it is unclear whether the varietal difference was due to a reduction of *C. cinctus* stem-mining injury in solid stemmed varieties, or whether solid stemmed varieties with the same amount of stem mining as hollow stemmed Reeder have better yield compensation.

Wheat seed quality is largely based on seed size, and *C. cinctus* herbivory also reduces seed size from infested stems. From a fitness perspective, smaller wheat seeds often do not germinate as consistently as larger seeds. For seed growers who harvest wheat with heavy field *C. cinctus* infestation, reduced average seed size may lead to lower quality for seed destined for sale to plant the next crop. From a grower's perspective, smaller seeds command lower market prices and price

drops are greater for lower seed size grades. *C. cinctus* herbivory reduces grain weight from mined stems and has the secondary impact of reducing seed quality of harvested grain.

In summary, the stress of larval stem mining on an individual wheat plant is reflected only in a mined stem: flag leaf photosynthesis reductions, slightly reduced stem height, and reductions in both grain weight and size. The environmental conditions a wheat plant experienced, especially phosphorus deficiency, explained more variation in grain weight reduction than did wheat variety for the impact of *C. cinctus* herbivory on main stem yield. Wheat yield decreases when absorbable soil phosphorus levels are reduced below a critical level, whereas at levels above this critical phosphorus concentration wheat yield is insensitive to higher soil phosphorus concentrations (Jackson et al. 1991, 1997; Havlin et al. 1999). Our greenhouse experimental procedure was sufficient to cause phosphorus deficiency in tested wheat plants, as reflected in flag photosynthetic reductions and subsequent seed mass reductions compared with unstressed plants. Of additional interest, *C. cinctus* mined (especially cut) stems were susceptible to the largest seed mass reductions when under phosphorus deficiency. Thus, our greenhouse results would support the importance of occasional phosphorus fertilization in areas where wheat is grown under high *C. cinctus* pressure, because low phosphorus not only reduces wheat yield but also magnifies yield loss due to *C. cinctus* stem mining. Water stress reduces wheat yield primarily by tiller reduction, and doubles the impact of *C. cinctus* on grain reductions. Wheat variety was somewhat important as infested Ernest and Choteau solid stems experienced somewhat smaller seed mass reductions than did the hollow stem Reeder variety. Thus, stem solidness may not only reduce stem lodging from *C. cinctus* by increasing mortality before cutting, but may also reduce stem-mining injury from surviving larvae both for grain weight and grain kernel size reductions. Our results indicate a second potential benefit of using solid stemmed wheat as a *C. cinctus* management tool. Because we have examined only main stem yield responses to *C. cinctus*, future studies will need to explore the impact of multiple stem infestation on the entire plant by this herbivore.

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