INFLUENCE OF *FUSARIUM* AND WHEAT STEM SAWFLY INFESTATION ON VOLATILE COMPOUNDS PRODUCTION BY WHEAT PLANTS

Dariusz Piesik[1,3]*, *Anna Wenda-Piesik*[2,3], *David K. Weaver*[3]
Tulio B. Macedo[3], *Wendell L. Morrill*[3]

[1] University of Technology and Life Sciences, Department of Applied Entomology
85-225 Bydgoszcz, Kordeckiego 20, Poland
[2] University of Technology and Life Sciences, Department of Plant Growth Principles and Experimental Methodology
85-225 Bydgoszcz, Kordeckiego 20, Poland
[3] Montana State University, Department of Land Resources and Environmental Sciences
334 Leon Johnson Hall, Bozeman, MT 59717-3120, USA

Received: January 1, 2009
Accepted: March 31, 2009

Abstract: Plants that were infested by the wheat stem sawfly emitted significantly increased amounts of the secondary metabolites linalool, linalool oxide, ß-farnesene, ß-caryophyllene, and 4-heptanone in comparison to uninfested plants. Wheat plants parasitized by *Fusarium* species also have emitted volatiles. The amount of volatiles released varied by infective species of *Fusarium* and volatile patterns varied over time for both sawfly infestation and pathogen infection. Plants that were stressed by both herbivory and pathogens emitted even greater amounts of certain volatiles. Surprisingly, larval sawfly mortality was much greater in plants infected by *Fusarium* species, and both mortality and the rate of volatile release varied by *Fusarium*. The role of these plant volatiles are further discussed in the context of defensive plant responses to *Fusarium* and sawfly infestation.

Key words: wheat, *Triticum aestivum*, volatiles, semiochemicals, odors, *Fusarium* spp., wheat stem sawfly

INTRODUCTION

The wheat stem sawfly, *Cephus cinctus* Norton (*Hymenoptera*: *Cephidae*), is a major pest of wheat, *Triticum aestivum* L, in the northern Great Plains of the United States and the Canada (Weiss *et al*. 1992; Weaver *et al*. 2004; Weaver *et al.* 2005). The annual losses caused by this insect exceeded $25 million in recent years in Montana (Weaver *et al*. 2004; Nansen *et al*. 2005). *C. cinctus* may reduce both harvest efficiency and head weight (Morrill *et al*. 1992; Nansen *et al*. 2005). Crop damage is also caused by other related species, including decreased net kernel weight, plus reduced yield and quality and has worsened in recent years (Huang *et al*. 2003), indicating that members of the *Cephidae* are a concern in many parts of the world. Understanding the chemical ecology of *C. cinctus* (Bartelt *et al*. 2002; Cossé *et al*. 2002) could lead to new, environmentally friendly approaches for reducing agricultural losses, especially since insecticides are ineffective against larvae inside the wheat stem and adult emergence is difficult to predict (Weiss *et al*. 1992; Morrill *et al*. 1992).

Fusarium graminearum Schwabe Gr1 and *Fusarium culmorum* (W.G. Smith) Sacc. are considered the primary causal agents of basal rot diseases in wheat in this temperate region (Wildermuth and McNamara 1994). *Fusarium equiseti* (Corda) Sacc. *sensu* Gordon is a cosmopolitan species that colonizes senescing plants and also parasitically infects wheat (Hall and Sutton 1998). These species cause disease that can be broadly classified as cortical rot, and can also be named foot, root, crown, stem, and dry rots. *F. graminearum* causes *Fusarium* head blight (FHB) in small grains worldwide (Burlakoti *et al*. 2007). Moreover, a number of fungal species are implicated in the FHB disease complex including *F. graminearum* and *F. culmorum* (Parry *et al*. 1995). The contamination of mycotoxins associated with head blight of wheat and other grains caused by *F. graminearum* is a chronic threat to crop, human and animal health throughout the world (Afshar *et al*. 2007).

Worldwide efforts are underway to reduce the use of the insecticides and alternative, often integrated, control strategies are being developed. Beneficial predators and parasitoids with their complex biology, elaborate interactions with other organisms, and importance in pest control, are fascinating subjects for ecological studies, including in crops (Francis *et al*. 2004). Plant volatiles induced by herbivory are frequently used as olfactory cues by foraging herbivores and their natural enemies, and thus have potential for control of agricultural pests (Williams *et al*. 2005).

Insect herbivory and pathogen infection are challenged by innate physical and chemical defenses and by

*Corresponding address:
piesik@utp.edu.pl

induced secondary metabolism (De Moraes *et al*. 2001; Cardoza *et al*. 2002, 2003; Rasmann *et al*. 2005). Plants emit volatile compounds as a product of their interactions with the biotic environment. Techniques for headspace collection of volatiles, in combination with facile gas chromatography-mass spectrometry analysis have significantly improved our understanding of the biosynthesis and release of plant volatiles (Tholl *et al*. 2006).

Manipulating plant volatile signals may increase effectiveness of natural enemies in pest management (Dicke and Sabelis 1988; Turlings *et al*. 1990; De Moraes *et al*. 1998; Thaler 1999; Kessler and Baldwin 2001; Reddy and Guerrero 2004). Volatile emissions as indirect defenses have been investigated for many years and understanding volatile emissions from wheat leaves may be useful in understanding of the attraction of insect pests to cereal crops (Buttery *et al*. 1985). Young, intact wheat plants release primarily 'characteristic' (Hatanaka 1993) green leaf volatiles and also emit terpenoids and ketones (Buttery *et al*. 1985). Some of these are implicated in attraction and repellency of wheat pests certain insects to undisrupted wheat plants. For example, certain volatile compounds from wheat and oat seedlings were determined to be behaviorally active, epideictic signals that resulted from aphid infestation (Quiroz and Niemeyer 1998a, b).

Recently, we have focused our research on the volatile responses of a wheat to mechanical wounding (Piesik *et al*. 2006) and endemic pathogen infection (Piesik *et al*. 2007) in the laboratory. Several wheat volatiles that were induced by mechanical wounding were also induced via infection by congeneric *Fusarium* species that caused varying amounts of cortical rot disease (Piesik *et al*. 2006, 2007). These studies have now been expanded using the same wheat variety as was used in the previous studies. This allows laboratory experimentation on organisms that coexist in two trophic levels in crops, as a model system. The purpose of these current studies was to include both wheat stem sawfly infestation and infection in this study to examine the interaction between these organisms and the subsequent impact on volatile emissions.

MATERIALS AND METHODS

Plant culture

Experiments were performed at the Plant Growth Center, Montana State University in 2002 and 2003. 'McNeal' spring wheat plants were planted and grown daily in a greenhouse with supplemental light and ambient humidity. The photoperiod was 16L:8D. The daytime temperature was 22±2°C and the overnight temperature was 18±2°C. Plants were grown two per pot in equal parts MSU PGC soil mix (equal parts of sterilized Bozeman Silt Loam soil: washed concrete sand and Canadian sphagnum peat moss) and Sunshine Mix 1 (Canadian sphagnum peat moss, perlite, vermiculite, and Dolmitic lime – Sun Gro Horticulture, Inc., Bellevue, Washington, USA). The plants were watered four times weekly, and fertilized with Peters® General Purpose Fertilizer (J.R. Peters Inc., Allentown, Pennsylvania, USA) at 100 ppm in aqueous solution twice each week as part of the regular watering schedule. Fertilizing commenced when the plants reached the three leaf stage.

Fusarium culture preparation

Cultures of *F. culmorum* (W.G. Smith) Sacc., *F. equiseti* (Corda) Sacc. Sensu Gordon and *F. graminearum* Schwabe Gr1 (*syn. pseudograminearum*) were used. These species were isolated from wheat stem sawfly larval cadavers, single-spore cultured, and deposited in a Montana State University collection on potato dextrose agar (PDA) slants. To prepare inoculum for use in these experiments, the stored cultures were rejuvenated on the PDA plates at 21–24°C for 14 days. Equal parts of barley and wheat grain were mixed (250 g total) and placed in water in 1 000 ml glass jars for 16 h. Excess water was removed and the grain was autoclaved for 30 min at 121°C. Grain was inoculated with three 1 diameter plug of *Fusarium* mycelium from the PDA culture and incubated for 21 days at 24°C (Dodman and Wildermuth 1987). The colonized grain was air-dried and ground in a laboratory mill to pass through a 1-mm sieve. 'McNeal' spring wheat was planted ata 3-cm depth in pots (1 210 cm^3) filled with sterilized soil and moistened to 37.5% water content (Wildermuth and McNamara 1994). Soil used in this experiment was a mixture of 1:1 MSU PGC mix and Sunshine Mix (Piesik *et al*. 2007). The dry, ground inoculum was applied as a layer in the soil that was placed 1-cm above the seed at rate of 1.4 g per pot. The moisture of the soil was maintained at 37.5% for the duration of the experiment.

Disease and rating

To determine the wheat infection due to the *Fusarium* spp., plants were examined once for the incidence of cortical rot at the Zadoks 59 wheat stage. The plant disease was subsequently assessed at maturity, after the plants were removed from the soil (one individual per each pot) according to the 0–4 scale described by Wildermuth and McNamara (1994). The visual assessment was made for to the control plants. Determination of the causal organism for each *Fusarium* infection was made for all stems in each treatment. Tissue from the subcrown area was sterilized, placed on PDA, and cultured for 30 days to identify the *Fusarium* species.

Infestation by wheat stem sawfly

Three *Fusarium*-infected plants and three control plants were subjected to oviposition by female wheat stem sawflies. The main stem from each pot was put in plastic cylinder. On the top and bottom of the plastic cylinder (9 cm long, 4.5 cm diameter), two holes were made to surround the inserted wheat stem. Two newly-emerged female sawflies were introduced in each cylinder. Infestation was assessed by stem dissection after the third volatile collection. The plants were subjected to oviposition two weeks before the collection began.

Volatile Collection System

The custom built apparatus (Analytical Research Systems, Inc., Gainesville, Florida, USA) used to collect volatiles (Piesik *et al*. 2006, 2007) featured a set of twelve glass volatile collection chambers that are open at one end to

enclose the growing plant. A flexible Teflon® sleeve was tape-sealed around the base of the main stem to prevent the collection of excess soil volatiles. The chambers were 40 diameter X 800 long. Volatiles were collected simultaneously from all six chambers. The plants were kept in the volatile chambers only while odors were collected. Each volatile collection chamber was fitted with manifold with 8 ports. Each port was fitted with threaded air inlet caps and threaded volatile collector ports, both with no. 7 ChemThread inlets (inner diameter 6.35 mm) using rubber O-rings. A volatile collector trap (6.35 OD, 76 mm-long glass tube; Analytical Research Systems, Inc., Gainesville, Florida, USA) containing 30 mg of Super-Q (Alltech Associates, Inc., Deerfield, Illinois, USA) adsorbent was inserted into each port, and sealed by the O-ring/ChemThread assembly. Purified, humidified air was delivered at a rate of 1.0 liter/min over the plants, and the flow and pressure were maintained by a vacuum pump.

The volatile collection system was computerized and had software inputs, which allowed two event controllers to switch solenoid switches off and on. These switches allowed the airflow of entrained volatiles to be switched from one port to another. This capability allowed for the programming sequential six-hour collections from each plant during photophase. Volatiles were collected from the main stem and the three large, uppermost leaves of each plant only. The volatile collection sequence (six hour collections) was initiated on Zadoks 32 (Zadoks *et al*. 1974), again at 2 days after, and again at four days after. For each collection interval, ten plants were collected: three with *Fusarium* and sawflies, two with *Fusarium*, three with sawflies only, and two controls. Specific treatment replicates for collection were assigned randomly every day and experiments were staged daily until the completion of the experiment. Additionally, one control chamber was collected each day. This control consisted of the airspace above of a pot containing soil only.

Analytical Methods

Volatiles were eluted from the Super-Q in each volatile collection trap with 225 µl of hexane. After this, 7 ng of decane was added as an internal standard. Volatiles were analyzed by coupled gas chromatography-mass spectrometry (GC-MS). The GC was an Agilent Technologies 6890 instrument fitted with a 30 m DB-1MS capillary column (0.25 ID, 0.25 µm film thickness; J & W Scientific, Folsom, CA, USA). The temperature program increased the chromatography oven temperature from 50°C to 280°C at 10°C/min. The MS instrument was an Agilent Technologies 5973. The identification of volatiles was verified with authentic standards purchased from commercial sources that had the same GC retention times and mass spectra.

Statistical Methods

All data was analyzed using the mixed models procedure (PROC MIXED) of SAS (SAS Institute 2001). Limitations due to the sampling capacity of the apparatus necessitated analysis of carry-over effects for structure due to time factors embedded in the experiment. Therefore, we used an analysis of variance (ANOVA) to address temporal variability in a type 3 test of fixed effects for carry-over by compound. The random effects in this mixed model were replicate (date). Because we did not observe any significant carry-over effect (Table 2), we were then able to perform a split-split-split plot analysis with the date of collection serving as the main plot and presence or absence of sawfly, presence or absence of fungus, and fungal species as successive subplots in an ANOVA for type 3 fixed effects. The random effects for this mixed model were replicate and the replicate by date interaction. For significant effects from this split-split-split plot ANOVA (Table 3), the means were separated using a Student test ($\alpha = 0.05$) which are shown in tables 4–6.

RESULTS

Infestation by the wheat stem sawfly and infection by the three *Fusarium* spp. was very reliable throughout the experiment (Table 1). In one experiment infestation was only seventy per cent, but infection occurred whenever the plants were inoculated. All plants inoculated with the *Fusarium* spp. had disease severity ratings of at least 2 on a 0–4 scale. There was considerable mortality of immature wheat stem sawflies in the stems inoculated by fungi.

F. graminearum had a severe impact with all stems having symptoms of the highest level of cortical rot. Disease appeared from the crown to the second node on the main stems and to above the second node on the tillers. Unexpectedly, all stems that were infested by wheat stem sawfly contained only cadavers. Insect infested plants that were previously inoculated with *F. graminearum* had more than one egg or small larval cadaver inside the stem. All cadavers had distinct symptoms of *Fusarium* infection (discolored and mycelium on their surface). Larvae in the uninfected stems were active, normally feeding, and occurred singly in each stem (Table 1).

Plants inoculated with *F. equiseti* showed symptoms of cortical rot. Plants disease ratings ranged from moderate to severe. Typically, disease symptoms were at the highest rating in the ranking scale. All exposed stems were infested with larval sawfly and all larvae occurring in the *F. equiseti* infected stems occurred singly and all had died. Uninfected stems were also singly infested, but there was 30% larval mortality in these controls (Table 1).

Wheat plants inoculated with *F. culmorum* had 100% infection, but the disease symptoms ranged from mild to only moderately severe. The lesions typically occurred as per the very first stage of disease progression and appeared uniformly on stems, but rotting had not begun. Living larvae occurred singly in most stems infected with *F. culmorum*, 30% of the stems contained only a few dead eggs. The sawfly infestation in uninfected plants reached 70%, but larval mortality was quite high at 50% (Table 1).

Selected volatile compounds produced by treated plants were generally emitted in significantly greater amounts compared to control plants, whether they were infested, infected, or both. There was no evidence of any significant carry-over effects from the experimental design for linalool, linalool oxide, ß-farnesene, ß-caryophyllene, and 4-heptanone (Table 2), indicating that the experimental design was sound for the comparison of treatment effects on the amounts of these compounds.

Table1. *Fusarium* infection and disease severity plus wheat stem sawfly infestation and larval mortality in experimental wheat plants

Treatment	*Fusarium* crown rot*		Sawfly infestation**	
	diseased plants [%]	severity rating (0–4)	infested plants [%]	larval mortality
F. graminearum + wheat stem sawfly	100	4	100	100
F. graminearum	100	4	–	–
Wheat stem sawfly	–	–	100	0
Control	–	–	–	–
F. culmorum + wheat stem sawfly	100	2	100	30
F. culmorum	100	3	–	–
Wheat stem sawfly	–	–	70	30
Control	–	–	–	–
F. equiseti + wheat stem sawfly	100	4	100	100
F. equiseti	100	3	–	–
Wheat stem sawfly	–	–	100	30
Control	–	–	–	–

* mean of three randomly chosen plants from pot
** total for three plants randomly chosen from pot

Table 2. By compound results of carryover analysis for effects due to randomly-assigned collection dates*

Compound	Effect	Num. DF	Den. DF	t-Value	P-Value
4-heptanone	species	2	76	6.2	0.003
	sawfly	1	76	3.76	0.056
	fungus	1	76	0.28	0.596
	carryover	1	76	1.48	0.228
B-caryophyllene	species	2	76	5.65	0.005
	sawfly	1	76	1.32	0.254
	fungus	1	76	0.01	0.920
	carryover	1	76	0.02	0.888
Linalool oxide	species	2	76	1.39	0.255
	sawfly	1	76	1.45	0.233
	fungus	1	76	0.12	0.733
	carryover	1	76	0.70	0.405
β-farnesene	species	2	76	1.43	0.245
	sawfly	1	76	1.39	0.243
	fungus	1	76	4.26	0.043
	carryover	1	76	3.25	0.075
Linalool	species	2	76	0.17	0.840
	sawfly	1	76	0.44	0.510
	fungus	1	76	0.46	0.499
	carryover	1	76	0.47	0.494

* type 3 test of fixed effects – the random effects in the mixed model were replicate(date of collection)

For 4-heptanone, two parameters (date of collection and fungal species) were significant (Table 3). None of the treatments and the possible interactions had a significant effect on two other compounds, linalool oxide and *β*-farnesene (data not shown).

For 4-heptanone, collection increased with time between collections, but a significantly larger amount (3-fold and 5-fold larger amounts, respectively) of this compound was collected between the 1st and 3rd and the 1st and 5th day of collection; 0.16, 0.54, and 0.80 ng, respectively (Table 4). The largest amount of 4-heptanone was recorded for *F. culmorum* (0.78 ng) in comparison to *F. equiseti* and *F. graminearum* at 0.27 and 0.44 ng, respectively (Table 4).

ß-caryophyllene collected in these aerations displayed a strong interaction between date of collection and fungal

Table 3. Results of the split-split-split plot analysis by collected volatile compound

Compound	Effect†	Num DF	Den DF	F-Value	P-Value
4-heptanone	D	2	6.81	12.57	0.005
	spp.	2	49.4	5.20	0.009
β-caryophyllene	D * spp.	4	45.8	5.37	0.009
linalool	D * S * F * spp.	4	49.5	3.28	0.018

* a Type
– s◎
mixed model are R and the interaction R*D

Table 4. Effect of date of collection or fungal species on the amount of 4-heptanone (ng) collected*

Date of collection		
Day 1 (Time 0)	Day 3 (48 h later)	Day 5 (96 h later)
0.16±0.07 a	0.54±0.09 b	0.80±0.17 b
Fungal species		
F. culmorum	*F. equiseti*	*F. graminearum*
0.78±0.18 a	0.27±0.06 b	0.44±0.09 b

*means followed by the same letter are not significantly different ($p \geq 0.05$)

Table 5. Effect of date of collection and fungal species on the amount of β-caryophyllene [ng] collected*

Date of Collection	Fungal species		
	*F. culmorum***	*F. equiseti***	*F. graminearum*
1 (time 0)	1.37±0.44 a	0.19±0.09 b	0.60±0.39 abX
3 (48 h later)	–	–	1.69±0.49 Y
5 (96 h later)	–	–	0.19±0.22 Z

* means followed by same letter within a row (a, b, c) or within a column (X, Y, Z) under each species are not significantly different ($p \geq 0.05$)
** "–" indicates that the compound was not found in these collections

Table 6. Effect of date of collection, sawfly species, and fungal species on the amount of linalool [ng] collected*

Species*	Date of collection											
	Day 1 (Time 0)				Day 3 (48 h later)				Day 5 (96 h later)			
	Sawfly											
	Present		Absent		Present		Absent		Present		Absent	
	Fungal											
	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence
F. c.	13.5±4.2	8.0±1.6 X	13.1±1.7 X	5.3±2.6 X	16.4±0.3	14.6±1.5	12.9±1.8 X	12.3±0.3 X	16.4±1.3	16.5±1.4	18.7±1.1	13.4±0.8 X
F. e.	13.2±8.9 a	1.0±0.3 bY	1.7±0.9 bY	0.8±0.1 bY	12.7±7.5 a	16.1±7.0 a	87.9±66.4 cY	7.3±0.7 aY	12.7±6.3 a	10.1±0.6 a	17.7±0.7 a	7.8±0.2a Y
F. g.	14.6±1.4	14.1±3.5 Z	24.7±13.9 Z	4.7±0.6 X	9.7±2.6	10.1±0.8	5.7±4.3 Z	4.8±3.7 Y	12.9±1.5	18.0±1.8	11.0±6.3	15.7±2.5 X

* fungal species are: *Fusarium culmorum* (*F. c.*), *Fusarium equiseti* (*F. e.*), and *Fusarium graminearum* (*F. g.*)
Means followed by different letters within a row (a, b, c) or within a column (X, Y, Z) under each parameter are significantly different

species (Table 3). Significant differences between *Fusarium* species were evident on the 1st day of collection. Wheat infested by *F. culmorum* produced the largest amount of ß-caryophyllene (1.37 ng) on the 1st day (Table 5). In contrast, for *F. equiseti*, the smallest collection occurred, at 0.19 ng (Table 5).

Variation in the amount of linalool collected in these aerations displayed a strong four way interaction between date of collection, sawfly infestation, fungal treatment and species of *Fusarium* (Table 3). Significant differences were noted only on day 1 for linalool collected after *F. equiseti* infection and sawfly infestation (Table 6), when compared to subsequent days of collection. In this collection, wheat plants infested by *F. equiseti* and sawflies released 13.2 ng of linalool, and this production remained similar in subsequent collections. Moreover, on the 1st day of collection time significant differences were observed among the plants infected by *Fusarium* species, but uninfested by wheat stem sawflies (1.7 ng for *F. equiseti* and 24.7 ng for *F. graminearum*).

Unexpectedly, at the 3rd collection time, the largest amount of linalool released by *F. equiseti* infected plants was noted in the absence of wheat stem sawfly infestation (87.9 ng).

DISCUSSION

This experiment resulted in very consistent levels of infestation and infection of wheat plants by the pathogenic fungi and the herbivorous insect, but the large amount of mortality of the insect immatures that occurred in plants infected by fungi was unexpected. The mortality could be due to pathogen-induced plant responses killing the insect larvae, or to unexpected insect pathology caused directly by the *Fusarium* spp. The stems were infected by the plant pathogenic species prior to oviposition, so there was no oviposition deterrence that was caused by induction by the fungal pathogen in these no-choice experiments. In choice experiments, repellency might have occurred, but this is the subject of future research.

The goal of this project was to build on the previous research that showed that volatile patterns induced by wounding or pathogen infection (Piesik *et al*. 2006; 2007) were pronounced, especially for both linalool and linalool oxide. In marked, but perhaps expected contrast, oviposition by a stem lumen feeding insect had minor influence on volatile production, perhaps because the larvae or eggs died quickly. Oviposition by this species causes a minor wound and feeding by small larvae on parenchyma lining the stem commences approximately 5 days after egg deposition (Weiss *et al*. 1992)

In this experiment the abundance of linalool, ß-farnesene, ß-caryophyllene, and 4-heptanone was visibly greater than for control plants, but significant variation could be explained by experimental treatments only for linalool, ß-caryophyllene, and 4-heptanone. In experiments studying peanut plant volatiles released in response to white mold infection disease and feeding by caterpillars showed no inhibition of the amounts of volatiles induced by fungi (Cardoza *et al*. 2002). However, the caterpillar damage in this system is profoundly greater than the oviposition wounds we studied. In Cardoza *et al*. (2002) concomitant infection and insect feeding elevated the emission of certain volatiles, which we observed for one fungal species immediately after oviposition. However, it is not possible to have a greater incidence of larval damage in our system because the eggs and neonates died and we have comparatively little injured. The results cannot be discussed in terms of potential semiochemical influence on the biological control of insects, other than that which might be unexpectedly caused by induction by the plant pathogenic fungi. This outcome is different than the subsequent study by Cardoza *et al*. (2003), which showed that the moth species preferred to oviposit on mold infected peanut plants, and that parasitoids preferred the concomitantly infected and infested plants. Although our oviposition events were no choice, it is still unusual that the eggs were deposited in stems infected by fungi where they had no chance for survival.

Secondary metabolites produced by plants have been reported to change development of other organisms (Chamberlain *et al*. 2000; Wang and Dorn 2003). This occurs by induction of host plants resulting in impacts on insect physiology and behavior, including reproduction, and by the plant defense responses to herbivory by insects (Reddy and Guerrero 2004). However, volatiles emitted from plants in response to insect damage can vary with insect feeding habitats (Rodriguez-Saona *et al*. 2003) and in our system insects died when encountering each fungal species, even though a only slight increase in a volatile terpenoid collection occurred after oviposition in stems infected with one fungal species. According to Francis *et al*. (2004) ß-farnesene might be a promising molecule for use as a biopesticide, while Petrescu *et al*. (2001) hypothesized that plants release ß-farnesene to habituate aphids, i.e., to disrupt their alarm-pheromone responses to reduce herbivory by increasing parasitoid or predator efficacy. We detected ß-farnesene in our system, but there were no treatment effects on its emission, because neither infection nor oviposition acted as triggers, especially given very limited insect feeding. Williams *et al*. (2005) reported that maize seedlings injured by *N. viridula* emitted higher amounts of the sesquiterpene (*E*)-beta-caryophyllene. Similar, detectable emission increases in (*E*)-beta-caryophyllene as the result of infestation was recorded by Rodriguez-Saona *et al*. (2002). We had increases in ß-caryophyllene in our system, but only due to the fungal infection. This contrasts with the findings of Cardoza *et al*. (2002) where caterpillar feeding increased emission while mold infection did not. The pattern Cardoza *et al*. (2002) observed for linalool is equivalent to that they observed for ß-caryophyllene, with insect induction, but no fungal induction. Once again, in our system involving only oviposition wounds, plus dying eggs or neonates, we observed the opposite. It is of importance to further investigate how plant induced secondary metabolites might be involved in the mortality of these young herbivores (Wang and Dorn 2003) and to also test for direct insect pathology in our system.

ACKNOWLEDGEMENTS

The USDA/CSREES Special Research Grant entitled "Novel Semiochemical– and Pathogen–Based Management Strategies for Wheat Stem Sawfly and Cereal Aphids" supported this project. Additional support was provided by the Montana Agricultural Experiment Station. The authors thank Megan Hofland and Micaela Buteler for technical assistance. Dariusz Piesik is grateful to the Batory and OECD Organization for help while stay in the USA.

REFERENCES

Afshar A.S., Mousavi A., Majd A., Renu A.G. 2007. Double mutation in tomato ribosomal protein L3 cDNA confers tolerance to deoxynivalenol (DON) in transgenic tobacco. Pakistan J. Biol. Sci. 10: 2327–2333.

Bartelt R.J., Cossé A.A., Petroski R.J., Weaver D.K. 2002. Cuticular hydrocarbons and novel alkenediol diacetates from wheat stem sawfly (*Cephus cinctus*): natural oxidation to pheromone components. J. Chem. Ecol. 28: 385–405.

Burlakoti R.R., Estrada R. Jr., Rivera VV., Boddeda A., Secor G.A., Adhikari T.B. 2007. Real-time PCR quantification and mycotoxin production of *Fusarium graminearum* in wheat inoculated with isolates collected from potato, sugar beet, and wheat. Phytopathology 97: 835–841.

Buttery R.G., Xu C.J., Ling L.C. 1985. Volatile components of wheat leaves (and stems): possible insect attractants. J. Agric. Food Chem. 33: 115–117.

Cardoza Y.J., Albron H.T., Tumlinson J.H. 2002. In vivo volatile emissions from peanut plants induced by simultaneous fungal infection and insect damage. J. Chem. Ecol. 28: 161–174.

Cardoza Y.J., Teal P.E.A., Tumlinson J.H. 2003. Effect of peanut plant fungal infection on oviposition preference by *Spodoptera exigua* and on host-searching behavior by *Cotesia marginiventris*. Environ. Entomol. 32: 970–976.

Chamberlain K., Pickett J.A., Woodcock C.M. 2000. Plant signaling and induced defence in insect attack. Mol. Plant Pathol. 1: 67–72.

Cossé A.A., Bartelt R.J., Weaver D.K., Zilkowski B.W. 2002. Pheromone components of the wheat stem sawfly: identification, electrophysiology, and field bioassay. J. Chem. Ecol. 28: 407–423.

De Moraes C.M., Lewis W.J., Paré P.W., Alborn H.T., Tumlinson J.H. 1998. Herbivore-infested plants selectively attract parasitoids. Nature 393: 570–573.

De Moraes C.M., Mescher M.C., Tumlinson J.H. 2001. Caterpillar-induced nocturnal plant volatiles repel nonspecific females. Nature 410: 577–580.

Dicke M., Sabelis M.W. 1988. How plants obtain predatory mites as bodyguards. Neth. J. Zool. 38: 148–165.

Dodman R.L., Wildermuth G.B. 1987. Inoculation methods for assessing resistance in wheat to crown rot caused by *Fusarium graminearum* Group 1. Aust. J. Agric. Res. 38: 473–486.

Francis F., Lognay G., Haubruge E. 2004. Olfactory responses to aphid and host plant volatile releases: (E)-ß-farnesene an effective kairomone for the predator *Adalia bipunctata*. J. Chem. Ecol. 30: 741–755.

Hall R., Sutton J.C. 1998. Relation of weather, crops, and soils variables to the prevalence, incidence, and severity of basal infections of winter wheat in Ontario. Can. J. Plant Pathol. 20: 69–80.

Hatanaka A. 1993. The biogeneration of green odour by green leaves. Phytochemistry 34: 1201–1281.

Huang X.G., Wang H.Q., Ge J.M., Gao H.C., Li C.X. 2003. Biology of the wheat stem sawfly and control countermeasures. Entomol. Knowledge 40: 515–518.

Kessler A., Baldwin I.T. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. Science 291: 2141–2144.

Nansen C., Payton M.E., Runyon J.B., Weaver D.K., Morrill W.L., Sing S.E. 2005. Preharvest sampling plan for larvae of the wheat stem sawfly, *Cephus cinctus* (*Hymenoptera: Cephidae*), in winter wheat fields. Can. Entomol. 137: 602–614.

Morrill W.L., Gabor J.W., Kushnak G.D. 1992. Wheat stem sawfly (*Hymenoptera: Cephidae*) damage and detection. J. Econ. Entomol. 85: 2413–2417.

Parry D.W., Jenkinson P., McLeod L. 1995. *Fusarium* ear blight (scab) in small grain cereals – a review. Plant Pathol. 44: 207–238.

Petrescu A.S., Mondor E.B., Roitberg B.D. 2001. Subversion of alarm communication: Do plants habituate aphids to their own alarm signals? Can. J. Zool. 79: 737–740.

Piesik D., Weaver D.K., Peck G.E., Morrill W.L. 2006. Mechanically injured wheat plants release greater amounts of linalool and linalool oxide. J. Plant Protect. Res. 46: 29–39.

Piesik D., Wenda-Piesik A., Weaver D.K., Morrill W.L. 2007. Influence of *Fusarium* crown rot disease on semiochemical production by wheat plants. J. Phytopathol. 155: 488–496.

Quiroz A., Niemeyer H.M. 1998a. Activity of enantiomers of sulcantol on apterae of *Rhopalosiphum padi*. J. Chem. Ecol. 24: 361–370.

Quiroz A., Niemeyer H.M. 1998b. Olfactometer-assessed responses of Aphid *Rhopalosiphum padi* to wheat and oat volatiles. J. Chem. Ecol. 24: 113–124.

Rasmann S., Kõllner T.G., Degenhardt J., Hiltpold I., Toepfer S., Kühlmann U., Gershenzon J., Turlings T.C.J. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434: 732–737.

Reddy G.V.P., Guerrero A. 2004. Interactions of insect pheromones and plant semiochemicals. Trends Plant Sci. 9: 253–261.

Rodriguez-Saona C., Crafts-Brandner S.J., Caňas L.A. 2003. Volatile emissions triggered by multiple herbivore damage: beet armyworm and whitefly feeding on cotton plants. J. Chem. Ecol. 29: 2539–2550.

Rodriguez-Saona C., Crafts-Brandner S.J., Williams L., Pare P.W. 2002. *Lygus hesperus* feeding and salivary gland extracts induce volatile emissions in plants. J. Chem. Ecol. 28: 1733–1747.

SAS Institute, 2001. SAS user's guide: statistics, version 8e. SAS Institute, Cary, NC, U.S.A.

Thaler J.S. 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature 399: 686–688.

Tholl D., Boland W., Hansel A., Loreto F., Röse U.S.R., Schnitzler J.P. 2006. Practical approaches to plant volatile analysis. Plant J. 45: 540–560.

Turlings T.C.J., Tumlinson J.H., Lewis W.J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250: 1251–1253.

Wang Q.H., Dorn S. 2003. Selection on olfactory response to semiochemicals from a plant-host complex in a parasitic wasp. Heredity 91: 430–435.

Weaver D.K., Sing S.E., Runyon J.B., Morrill W.L. 2004. Potential impact of cultural practices on wheat stem sawfly (*Hymenoptera: Cephidae*) and associated parasitoids. J. Agr. Urban Entomol. 21: 271–287.

Weaver D.K., Nansen C., Runyon J.B., Sing S.E., Morrill W.L. 2005. Spatial distributions of *Cephus cinctus* Norton (*Hymenoptera: Cephidae*) and its braconid parasitoids in Montana wheat fields. Biol. Contr. 34: 1–11.

Weiss M.J., Morrill W.L., Reitz L.L. 1992. Wheat stem sawfly (*Hymenoptera: Cephidae*) revisited. Am. Entomol. 38: 241–245.

Wildermuth G.B., Mcnamara R.B. 1994. Testing wheat seedlings for resistance to crown rot caused by *Fusarium graminearum* Group 1. Plant Dis. 78: 949–953.

Williams L., Rodriguez-Saona C., Pare P.W., Crafts-Brandner S.J. 2005. The piercing-sucking herbivores *Lygus hesperus* and *Nezara viridula* induce volatile emissions in plants. Arch. Insect Biochem. 58: 84–96.

Zadoks J.C., Chang T.T., Konsak C.F. 1974. A decimal code for growth stages of cereals. Weed Res. 14: 15–21.

POLISH SUMMARY

WPŁYW *FUSARIUM* SPP. I ŹDZIEBLARZA PSZENICZNEGO NA PRODUKCJĘ LOTNYCH METABOLITÓW WYDZIELANYCH PRZEZ PSZENICĘ

Rośliny zaatakowane przez ździeblarza pszenicznego emitują znacząco większe ilości wtórnych metabolitów takich jak: linalol, tlenek linalolu, ß-farnezen, ß-kariofilen i 4-heptanon, w porównaniu do roślin zdrowych. Także rośliny pszenicy zainfekowane przez *Fusarium* spp. emitują lotne związki, które różnią się jakościowo i ilościowo od roślin nieuszkodzonych. Ilości uwolnionych lotnych związków różnią się w zależności od gatunku *Fusarium*, a także w zależności od czasu jaki upłynął od momentu infekcji, zarówno w odniesieniu do patogena grzybowego, jak i ździeblarza pszenicznego. Rośliny zainfekowane przez patogena grzybowego i zaatakowane przez szkodnika wydzielają nawet większe ilości lotnych związków. Niespodziewanie śmiertelność larw była większa w odniesieniu do roślin zainfekowanych przez *Fusarium*. Rola lotnych związków powinna być dalej badana w kontekście systemu obronnego rośliny pszenicy w stosunku do *Fusarium*.