



Joint effects of biotic and abiotic stressors on winter wheat suppression of *Bromus tectorum*

N ORLOFF*, Z J MILLER†, F D MENALLED*, M E BURROWS†
& J M MANGOLD*

*Land Resources and Environmental Sciences Department, Montana State University, Bozeman, MT, USA, and †Plant Sciences and Plant Pathology Department, Montana State University, Bozeman, MT, USA

Received 2 November 2012

Revised version accepted 31 January 2013

Subject Editor: Karen Bailey, AAFC, Canada

Summary

In winter wheat systems in the Northern Great Plains of the United States, *Bromus tectorum* and wheat streak mosaic virus (*WSMV*) commonly co-occur. While independent effects of these pests on wheat yields have been well documented, to our knowledge, no study has investigated whether *WSMV* modifies interactions between wheat and *B. tectorum*. Furthermore, the impact that environmental stressors such as nutrient availability have on these interactions has not been addressed. We conducted a randomised split-plot field study over 2 years to investigate the effects of *WSMV* and nitrate (N) availability on winter wheat suppression of *Bromus tectorum*. The study included four N treatments (10–19, 20–31, 31–84, and 85–207 kg ha⁻¹) and two *WSMV* treatments (mechanically inoculated or control). Increasing soil N increased the susceptibility of wheat to *WSMV* infec-

tion. In 2009, wheat in the lowest and highest N levels had 24% and 65% of plants infected respectively. However, regression analysis indicated that interactive effects of wheat competition, N and *WSMV* did not play a consistent role in *B. tectorum* growth. Specifically, the effect of both wheat density and distance from row on *B. tectorum* biomass remained constant across inoculation treatments, suggesting that wheat inoculated with *WSMV* suppressed *B. tectorum* as effectively as healthy wheat. Furthermore, wheat had a greater impact on *B. tectorum* growth in higher N environments, even though incidence of *WSMV* infection in wheat was highest. Overall, our results suggest that *WSMV* infection may not change the ability of wheat to suppress *B. tectorum*.

Keywords: wheat streak mosaic virus, crop-weed competition, crop stress, weed management, multi-trophic interactions, crop pathology, disease.

ORLOFF N, MILLER ZJ, MENALLED FD, BURROWS ME & MANGOLD JM (2013). Joint effects of biotic and abiotic stressors on winter wheat suppression of *Bromus tectorum*. *Weed Research* **53**, 192–200.

Introduction

Traditional agricultural weed management relies on herbicides or tillage to reduce the spread and impact of undesired species. However, dependence on tillage and herbicides has resulted in soil erosion, proliferation of herbicide resistant weeds, decreased crop yields due to herbicide carryover effects, and surface and groundwater contamination (Liebman, 2001).

Adoption of ecologically based approaches is an important step towards sustainable weed management (Shennan, 2008). In agricultural settings, one ecologically based weed management approach is to maximise the competitive effect of the crop to minimise yield losses and weed spread (National Research Council, 2010). Practices for increasing crop competitive ability include using competitive cultivars, planting at increased densities, decreasing row spacing and

Correspondence: Noelle Orloff, Land Resources and Environmental Sciences Department, Montana State University, P.O. Box 173120, Bozeman, MT, 59717, USA. Tel: (+1) 406 994 6297; Fax: (+1) 406 994 3933; E-mail: noelleorloff@gmail.com

employing appropriate fertility strategies (Liebman & Mohler, 2001; Mohler, 2001). However, the goal of increased crop competitive ability is complicated by multiple environmental and biological stressors that alter weed and crop performance and interactions (Schroeder *et al.*, 2005). Furthermore, research usually focuses on a single pest or stressor and may result in conflicting management recommendations that do not achieve desired results (Norris, 2005; Schroeder *et al.*, 2005).

Assessing interactive effects of pests and environmental variables on weed performance and weed-crop competition is required to develop ecologically based pest management programmes (Louda *et al.*, 1990; National Research Council, 2010). For example, competition between crops and weeds may be altered if a shared pathogen has different impacts on the competing species (Alexander & Holt, 1998; Schroeder *et al.*, 2005). Impacts of pathogen infection on crop vigour are often well documented, but the response of weed species to plant diseases is rarely considered, despite the potential role of disease in mediating crop-weed interactions (Schroeder *et al.*, 2005). Weed species are usually less susceptible than crops to crop pathogens, and infected crops may be less able to suppress weed growth and be more susceptible to weed interference (Alexander & Holt, 1998).

Bromus tectorum L. (downy brome or cheatgrass) and wheat streak mosaic virus (*WSMV*; genus *Tritimo-virus*) are co-occurring pests of winter wheat (*Triticum aestivum* L.) in the Northern Great Plains (NGP) of the United States. *Bromus tectorum* is a problematic winter annual grassy weed in winter wheat fields due to similar life histories, widespread adoption of conservation tillage techniques and broadcast applications of nitrogen fertiliser (Morrow & Stahlman, 1984; Blackshaw, 1994). In a continuous winter wheat cropping system, *B. tectorum* can form dense stands with densities increasing from 24 to 940 plants m⁻² over 5 years (Blackshaw, 1994). *Bromus tectorum* also substantially decreases winter wheat yield. For example, densities of 24, 40 and 65 *B. tectorum* plants m⁻² caused 10%, 15% and 20% reductions in wheat yield respectively (Stahlman & Miller, 1990).

Wheat streak mosaic virus is the most common cause of viral disease of cereals in the NGP (Burrows *et al.*, 2009). The disease is vectored by the wheat curl mite (*Aceria tosichella* Keifer), and both vector and virus occur on a diverse set of grass species, including wheat and *B. tectorum* (Duffus, 1971; Christian & Willis, 1993). Infection by *WSMV* reduces wheat yields by interfering with chloroplast development (Brakke *et al.*, 1988) and inhibits root growth, leading to reduced water uptake (Price *et al.*, 2010). In infected

fields, yield loss due to *WSMV* can approach 100% in extreme cases (Atkinson & Grant, 1967). Because *B. tectorum* is less susceptible to *WSMV* infection than winter wheat (Ito *et al.*, 2012), the disease could modify competitive interactions between the two species. To our knowledge, no study has addressed whether *WSMV* modifies interactions between wheat and *B. tectorum* and the impact that environmental stressors such as nutrient availability have on these interactions.

Understanding how soil fertility and disease alter interactions between *B. tectorum* and winter wheat is critical to design effective weed management programmes. In cropping systems, nitrogen (N) fertiliser is the most common input used to increase crop yield, but N availability also impacts weed-crop competitive interactions and plant disease dynamics (Zimdahl, 2004; Dordas, 2008). First, fertilisation can have unintended consequences if weeds are more responsive than the crop to N enrichment, making them more competitive under elevated N conditions (Liebman & Mohler, 2001). For example, *B. tectorum* growth has been found to be more responsive than wheat to N enrichment; when soil N concentrations were increased from 0 to 240 mg N kg⁻¹ soil, wheat and *B. tectorum* shoot biomass increased by about 150% and 250% respectively (Blackshaw *et al.*, 2003). The effect of *WSMV* on crops, and thus crop-weed interactions, may also change with fertiliser strategies. Plant susceptibility to disease often depends on N, and infections from obligate pathogens such as *WSMV* are often more severe with higher N availability (Dordas, 2008).

In a field study, we evaluated the individual and joint impact of wheat competition, infection by *WSMV* and N availability on *B. tectorum* biomass. These factors are commonly encountered and jointly manipulated in small-grain cropping systems, but are usually investigated singly, although they may interact in important ways. In our analysis, we focused on determining whether interactive effects of wheat competition, *WSMV* and N were important in determining *B. tectorum* growth. Specifically, we hypothesised that *WSMV* infection and N enrichment would interact with wheat density and distance from row to impact *B. tectorum* biomass.

Materials and methods

Experimental design

This study was conducted during the 2009 and 2010 growing seasons at the Montana State University Post Research Farm, Bozeman, MT, USA (45°40'29" N,

111°09'14"W, 1423 m elevation). Long-term average annual precipitation (1966–2010) at the site is 416 mm, and total precipitation in 2009 and 2010 was above average at 441 and 454 mm respectively (Table 1; WRCC, 2008). Soils are classified as Amsterdam-Quagle Silt Loam (NRCS, 2008). The entire site had been tilled fallow for at least 2 years prior to the start of this research.

A randomised split-plot design with four blocks was used. Nitrogen treatments were randomly assigned to main plots (5.0 m × 36.6 m), and *WSMV* inoculation treatments were randomly assigned to subplots (5.0 m × 1.2 m). In 2009, there were two high N and two low N main plots per block, while in 2010, we had one main plot per block for each of four N treatments. Main plot and subplot locations were randomly assigned for each year. On 8 October 2008 and 6 October 2009, the entire site was planted with winter wheat (var. Neely) at 67 kg ha⁻¹ and 25 cm row spacing utilising a no-till drill. Throughout the study, broad-leaved weeds were controlled with pyrasulfotole + bromoxynil (Huskie®, Bayer CropScience LP) as needed at recommended rates.

To estimate N availability prior to planting, soil cores (three per block) were taken on 29 August 2008 to a depth of 91 cm, and N was analysed by AgVise laboratories (Northwood, North Dakota). Based on the results, urea fertiliser was applied to the main plots using a handheld broadcast spreader in September 2008 to reach two target N levels [95 kg ha⁻¹ (no fertiliser added) and 291 kg ha⁻¹ (high)]. The high N level corresponded to recommended rates for the region (Jacobson *et al.*, 2003). To assess N availability during the growing season, five soil cores per plot were taken to a depth of 45 cm on 9 June 2009 and composited for analysis as described previously. These

samples revealed that due to pre-existing variation in N among subplots, N ranged between 11 and 207 kg ha⁻¹. Finally, we obtained 45 cm soil cores to evaluate post-harvest N levels on 17 September 2009. Based on these values, urea fertiliser was applied to main plots in April 2010. Based on N values observed in 2009, four target N levels (17 kg ha⁻¹ (ambient), 122, 244 and 366 kg ha⁻¹) were randomly assigned to the main plots for the 2010 trial. Soil tests for N were taken again during the second growing season on 9 June 2010 (five per subplot) to a depth of 45 cm and analysed as described previously.

To secure adequate *B. tectorum* density, locally collected seeds were hand-broadcasted in all subplots on 16 September 2008 at a density of 2500 seeds m⁻². In 2009, *B. tectorum* produced seeds within subplots, providing the seed required for the second year trial. To minimise the impact of the observed relationship between N available in 2009 and seed inputs on *B. tectorum* abundance, we added 3100 seeds m⁻² from local *B. tectorum* populations on 18 September 2009 to subplots that had less than 34 kg ha⁻¹ N during the summer 2009 sampling.

On 21 May 2009 and 19 May 2010, half of the subplots were inoculated with *WSMV*. We chose this timing because in cold climates, mechanically inoculating winter wheat with *WSMV* in the spring causes more severe infections than fall inoculation (Ito, 2011). Inoculation procedures were similar to Price *et al.* (2010). Briefly, trays of a susceptible wheat variety were grown in the glasshouse and repeatedly inoculated with *WSMV* strain Conrad I (Ito *et al.*, 2012). Infected wheat tissue was macerated in a blender with phosphate-buffered saline (PBS 136.9 mM NaCl, 8.1 mM Na₂HPO₄, 1.5 mM of KH₂PO₄ and 2.9 mM KCl, pH 7.2) (1:10 weight in g of tissue to volume in mL of

Table 1 Monthly precipitation and average air temperatures in 2009, 2010 and long-term averages (1966–2010; 44 years) at the Montana State University Post Research Farm, Bozeman, MT, USA (WRCC, 2008)

Month	Mean temperature (C)			Precipitation (mm)		
	2009	2010	44 years mean	2009	2010	44 years mean
January	-2.8	-4.3	-4.9	3.8	13.7	14.0
February	-0.3	-2.8	-2.6	9.7	7.9	13.0
March	-0.7	2.9	1.3	38.9	25.4	26.2
April	5.1	6.2	5.9	71.9	37.8	42.9
May	12.1	8.3	10.7	42.4	85.9	68.1
June	14.1	13.9	14.7	66.5	119.1	72.1
July	18.5	18.6	18.8	70.9	9.9	34.8
August	18.2	18.1	18.2	39.6	45.2	31.8
September	17.6	13.9	13.3	13.7	40.4	36.1
October	3.7	10.2	7.2	46.5	17.8	36.1
November	0.8	-2.3	-0.1	30.5	35.3	22.6
December	-9.2	-5.3	-4.8	7.1	16.0	14.5
Total Annual Precip.				441.5	454.4	412.0

buffer) to make inoculum. The inoculum was mixed with silicon carbide powder (320 grit, 0.5 g per 1 L buffer) and applied in the field at a rate of 3 L plot⁻¹ using an air compressor and sprayed with paint guns at 550 kPa. To check that there were no vileriferous wheat curl mites spreading *WSMV* outside of mechanically inoculated treatment plots, we monitored the site for mites and our control plots for unintended spread of the virus. We found no evidence that *WSMV* was spread outside of treatment plots. In total, in 2009, we implemented two target N treatments and two *WSMV* treatments replicated 12 times for a total of 48 subplots. In 2010, we had four target N treatments and two *WSMV* treatments replicated five times for a total of 40 subplots.

Plant community sampling

Within each subplot, we randomly placed three (2009) or five (2010) 0.1 m² rings (35.68 cm diameter) to define competitive neighbourhoods. About half of the rings were centred on the wheat row, while the others were centred on the inter-row. This sampling arrangement allowed us to measure target *B. tectorum* individuals growing both close to and far from the wheat row. On 28 May 2009 and 15 July 2010, wheat density was counted within rings to investigate how the per-capita effect of wheat on *B. tectorum* changed with N and *WSMV* treatments. *Bromus tectorum* density was measured simultaneously to be used as a covariate. In the centre of each ring, up to three *B. tectorum* target individuals were harvested when all seeds were mature and prior to seed shatter. In 2009, *B. tectorum* matured simultaneously in all treatments, so biomass was collected at the same time (14 July). In 2010, plants matured more quickly in the two lowest N treatments. Accordingly, we harvested all *B. tectorum* at the same phenological stage but at different times (16 July for the two lowest N treatments and 28 July for the two highest N treatments). Distance of each harvested *B. tectorum* individual to closest wheat row was measured at the time of harvest, as previous work (Stahlman & Miller, 1990) demonstrated that *B. tectorum* size increases proportionally to its distance from winter wheat. In total, we harvested 370 and 409 *B. tectorum* plants in 2009 and 2010 respectively. These plants were dried to constant biomass in a 60°C oven and weighed to the nearest 0.01 g.

Detection of wheat streak mosaic virus

Wheat and *B. tectorum* were evaluated for *WSMV* infection when wheat in inoculated subplots began

to show symptoms of systemic infection, including chlorosis and streaking (24 June 2009 and 9 June 2010). Ten wheat leaves from 10 different plants (both symptomatic and asymptomatic) were collected in inoculated subplots, and one leaf was collected from non-inoculated subplots (controls). Samples were collected outside of the rings. *Bromus tectorum* leaves from each inoculated subplot were collected (three and 10 leaf samples per subplot in 2009 and 2010 respectively), and one leaf sample from non-inoculated subplots was also collected (controls). Samples were tested for *WSMV* using a double antibody sandwich enzyme-linked immunosorbant assay (ELISA) using methods identical to Ito *et al.* (2012). To determine the incidence of *WSMV* infection at the subplot level in both wheat and *B. tectorum*, we compared absorbance values measured with a microplate reader (SpectraMax Plus; Molecular Devices, Sunnyvale, CA) at 405 nm between samples from inoculated subplots and controls. Absorbance of non-inoculated plants was used to create a cut-off value for infected individuals by calculating a 99% confidence interval around the mean absorbance of controls on each plate. Samples with absorbance values above the upper boundary of the confidence interval were considered infected.

Statistical analysis

Wheat and *B. tectorum* densities were compared between years using *t*-tests. Wheat and *B. tectorum* disease incidence was compared between N levels using ANOVA with incidence of infection as the response variable and year and N level as predictors. Within each species, differences in mean *WSMV* incidence between years and N levels were further investigated using Tukey's HSD (Honestly Significant Difference) *post hoc* tests.

Changes in individual *B. tectorum* biomass as a function of growing conditions were modelled using a linear mixed effects regression model following Pinheiro and Bates (2000). The analysis was conducted using R software including the nlme, gmodels and LMERConvenienceFunctions packages (R Development Core Team, 2008; Pinheiro *et al.*, 2011; Tremblay & Ransijn, 2012). Fixed effects were N level and *WSMV* inoculation as categorical variables, distance from row and wheat density as continuous variables and *B. tectorum* density as a covariate. The random component of the model included year, N within year (whole plots), *WSMV* within N (subplots) and ring within subplot. This nested random structure allowed generalisation over the 2 years and also accounted for the split-plot design (Pinheiro & Bates, 2000).

Bromus tectorum individual biomass data were natural log-transformed to normalise the distribution of the response variable. Further, because of the patchy background levels of N found in 2009 during soil testing, we did not use the assigned N treatment levels for data analysis. Instead, we used results from soil tests taken during the growing season to create categorical variables for N. These categorical variables were determined by combining all *B. tectorum* individuals and associated soil N measurements for the 2 years and dividing them into four equally sized groups. The N categories used for data analysis were; 1 (10–19 kg ha⁻¹, *n* = 202), 2 (20–31 kg ha⁻¹, *n* = 201), 3 (32–84 kg ha⁻¹, *n* = 197) and 4 (85–207 kg ha⁻¹, *n* = 174). This variable describes N present in the soil during the growing season, rather than amendments applied. Because soil N was tested in June of each growing season, most of the available N had been taken up (Malhi *et al.*, 2006), which explains the relatively low values.

In our analysis, the slope of the relationship between distance from row and *B. tectorum* individual biomass was interpreted as the percentage decrease in ln(*B. tectorum* biomass) for each cm it occurred from the wheat row. Similarly, the slope of the relationship between wheat density and ln(*B. tectorum* biomass) was interpreted as the percentage decrease in ln(*B. tectorum* biomass) for each additional wheat plant in the ring. We expected the relationship between distance from row and/or wheat density and ln(*B. tectorum* biomass) to change if wheat competitive ability was modified by the N and *WSMV* treatments. Thus, we tested for a three-way interaction between distance from row or wheat density, N and *WSMV* to determine whether N and *WSMV* changed either relationship. We simplified the model based on log-likelihood ratios (Tremblay & Ransijn, 2012). We checked for equal variance among and within groups using diagnostic plots (Pinheiro & Bates, 2000) and found no evidence of unequal variance.

Results

Wheat density and *B. tectorum* density differed between years (Table 2). We observed substantial wheat winterkill in 2010 due to snow mould (*Microdochium* [*Fusarium*] *nivale*) which likely influenced variation in wheat density (Table 2). As expected, wheat had higher incidence of *WSMV* infection than *B. tectorum* (Ito *et al.*, 2012). Incidence of *WSMV* infection differed between years and among N levels. Wheat incidence was about 20% higher in 2009 compared with 2010 across N levels (*P* < 0.001, Table 3). Additionally, wheat grown in higher N levels had higher

Table 2 Means, standard deviations, maximum values and minimum values for wheat density and *Bromus tectorum* density within competitive neighbourhoods (0.1 m² rings)

Year	Wheat density				<i>B. tectorum</i> density			
	Mean	SD	Max	Min	Mean	SD	Max	Min
2009	11.03 ^a	4.96	35	0	11.81 ^a	7.79	51	1
2010	14.45 ^b	8.60	63	1	20.29 ^b	15.34	94	1

Within plant species, superscript letters indicate differences in mean densities per year based on *t*-tests ($\alpha = 0.05$).

Table 3 Mean percentage incidence of infection (standard deviation) for winter wheat mechanically inoculated with wheat streak mosaic virus

Year	N levels			
	1	2	3	4
2009	24 (12) ^a	38 (13) ^b	47 (23) ^b	65 (15) ^c
2010	9 (14) ^a	18 (13) ^b	29 (20) ^b	37 (23) ^c

Nitrate levels (N) were 1 (10–19 kg ha⁻¹), 2 (20–31 kg ha⁻¹), 3 (31–84 kg ha⁻¹) and 4 (85–207 kg ha⁻¹). Within year, superscript letters indicate differences in treatment means based on Tukey's HSD *post hoc* tests.

Table 4 Results from ANOVA assessing the effect of nitrate level (N), wheat streak mosaic virus (*WSMV*) inoculation, wheat density, *Bromus tectorum* density and distance from wheat row on response variable ln(individual *B. tectorum* biomass) in the most parsimonious linear mixed effects model

	Degrees of freedom (d.f.)	Error d.f.	F-value	P value
Intercept	1	446	172.0	<0.001
Wheat density	1	224	31.53	<0.001
<i>B. tectorum</i> density	1	224	12.10	<0.001
Distance	1	446	18.62	<0.001
N	3	73	25.21	<0.001
<i>WSMV</i>	1	73	1.249	0.440
Wheat density × N	3	224	0.603	0.048
N × <i>WSMV</i>	3	73	0.020	0.015

Nitrate levels were 1 (10–19 kg ha⁻¹), 2 (20–31 kg ha⁻¹), 3 (31–84 kg ha⁻¹) and 4 (85–207 kg ha⁻¹).

infection rates in both years (*P* < 0.001, Table 3). Incidence of *WSMV* infection in *B. tectorum* was low (3%, data not presented), regardless of year or N level.

Inoculation with *WSMV* modified the effect of N on *B. tectorum* biomass (N × *WSMV* interaction, *P* = 0.015, Table 4). In general, increased N led to increased *B. tectorum* biomass (*P* < 0.001, Table 4).

However, according to *post hoc* Wald tests, *WSMV* inoculation increased *B. tectorum* biomass with N level 1 and decreased it in N level 3 ($P = 0.036$ and 0.046 respectively Fig. 1). With N level 1, *B. tectorum* median biomass was 0.23 g in non-inoculated plots and 0.36 g in inoculated plots (Fig. 1), an increase of 56%. With N level 3, *B. tectorum* median biomass was 1.02 g in non-inoculated plots and 0.65 g in inoculated plots (Fig. 1), a decrease of 36%. We did not detect a difference between inoculated and non-inoculated *B. tectorum* biomass in N levels 2 or 4 ($P = 0.676$ and 0.157 respectively Fig. 1).

We hypothesised that the effects of wheat competition (distance from row and wheat density) on *B. tectorum* biomass would be modified by N availability and *WSMV* inoculation. Our results partially support this hypothesis. Distance from wheat row directly affected *B. tectorum* biomass. *Bromus tectorum* biomass increased by $4.5 \pm 1.1\%$ with each cm from the wheat row ($P < 0.001$, Table 4). However, the effect of distance from row did not change with either the N or

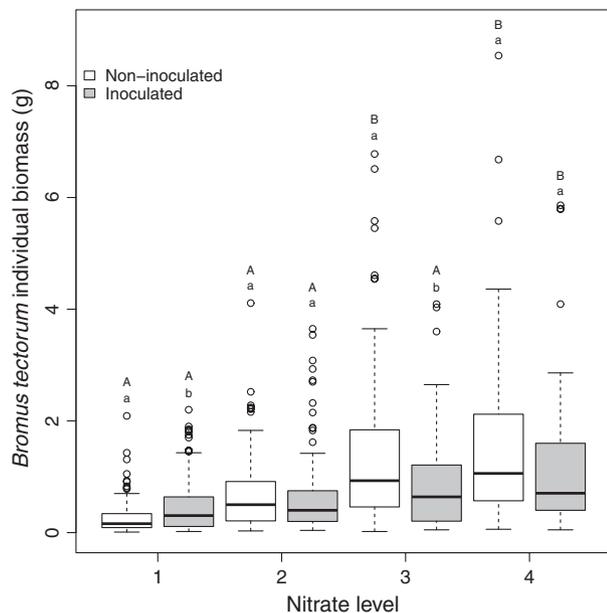


Fig. 1 Impact of wheat streak mosaic virus (*WSMV*) and nitrate (N) level on untransformed individual *Bromus tectorum* biomass (g). N levels were; 1 (10–19 kg ha⁻¹), 2 (20–31 kg ha⁻¹), 3 (31–84 kg ha⁻¹) and 4 (85–207 kg ha⁻¹). Bold horizontal lines represent median *B. tectorum* biomass for each N × *WSMV* treatment combination. The bottom and top of the boxes show the 25th and 75th percentiles respectively and represent the middle 50% of the data in each group. Error bars show 1.5 times the interquartile range of the data, and open circles represent observations outside this range. Letters separate medians that are significantly different according to *post hoc* Wald tests ($\alpha = 0.05$). Upper case letters represent differences among N level within *WSMV* treatment, and lower case letters represent differences between *WSMV* treatments within N level.

WSMV treatments, as shown by the lack of a distance from row × N or distance from row × *WSMV* interaction ($P = 0.820$ and 0.934 respectively data not presented). In general, the effect of increasing wheat density on *B. tectorum* biomass increased with N enrichment ($P = 0.048$, Table 4, Fig. 2). According to *post hoc* Wald tests, the effect of wheat density on *B. tectorum* biomass was similar across N levels 1, 2 and 3, but increased in N level 4 (Fig. 2). *WSMV* did not alter effects of wheat density (wheat density × *WSMV* interaction, $P = 0.433$, data not presented), suggesting that the competitive ability of wheat was not modified by *WSMV*.

Discussion

Interactions between biotic and abiotic factors can impact weed performance and crop-weed interactions (Norris, 2005; Schroeder *et al.*, 2005; Wisler & Norris, 2005). Knowledge of these effects may allow managers to promote conditions that favour crop competitive ability and reduce weed pressure (National Research Council, 2010). To better understand these potential impacts in a winter wheat pest complex, we investigated the effects of *WSMV* infection, nutrient availability and winter wheat competition on *B. tectorum* performance. Contrary to our hypothesis, interactive

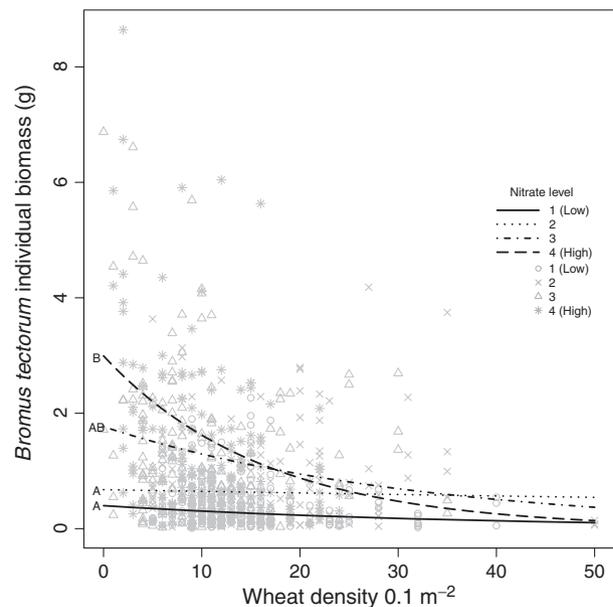


Fig. 2 *Bromus tectorum* individual biomass response to increasing wheat density within competitive neighbourhoods (0.1 m² rings) in each of the four nitrate levels [1 (10–19 kg ha⁻¹), 2 (20–31 kg ha⁻¹), 3 (31–84 kg ha⁻¹) and 4 (85–207 kg ha⁻¹)]. Points represent observed values, and lines represent fitted values. Letters separate slopes that are significantly different according to *post hoc* Wald tests ($\alpha = 0.05$).

effects of the measured variables did not appear to play a consistent role in *B. tectorum* biomass production.

The incidence of *WSMV* infection in wheat and *B. tectorum* in our study was representative of *WSMV*-infected fields in the Northern Great Plains (Ito *et al.*, 2012). For winter wheat and *B. tectorum*, we achieved mean incidence rates of 34% and 3% respectively. However, incidence of infection in wheat across N levels was about 20% higher in 2009 compared with 2010. This discrepancy may be partially due to differences in weather during the month after we inoculated plots. May 2009 was warmer and drier than average, and May 2010 was cooler and wetter than average. Temperature and moisture can each impact plant susceptibility and the extent of infection, and the effect of these factors on viruses is unpredictable (Agrios, 2005).

We observed inconsistent direct effects of *WSMV* inoculation on *B. tectorum* growth. In very low N environments (N level 1), *B. tectorum* in inoculated plots grew larger than *B. tectorum* in non-inoculated plots, while the opposite occurred in relatively higher N level 3 plots. In the remaining two N levels (N levels 2 and 4), there was no detectable effect of inoculation on *B. tectorum* performance. Incidence of infection for wheat was lowest in the low N environment, meaning that it is unlikely that wheat infection caused *B. tectorum* to be released from competition only in that N level. While we do not have a satisfactory explanation for the results, the consistency observed across years suggests that future studies could concentrate on the mechanisms driving these observations.

Inoculated wheat suppressed *B. tectorum* as effectively as healthy wheat. Despite the increasing disease incidence with increasing N observed in wheat, the effect of both wheat density and distance from row on *B. tectorum* biomass remained constant between *WSMV* inoculation treatments. Further, within the *WSMV* inoculation treatment, wheat density had a greater impact on *B. tectorum* in higher N environments, even though incidence of *WSMV* infection in wheat was highest in these high N environments. These results were unexpected, as previous studies have shown that *WSMV* infection reduces root growth and resource uptake in winter wheat (Price *et al.*, 2010). Thus, infected wheat should use fewer resources, making more available to *B. tectorum* and allowing it to accumulate more biomass. This hypothesis was supported by Williams and Patoky (2012), who found that increasing incidence of maize dwarf mosaic in sweet corn decreases its ability to suppress the common weed *Panicum miliaceum* L. (wild-proso millet). The relative timing of competition

between wheat and *B. tectorum* versus the impacts of *WSMV* on wheat may help explain this discrepancy. *Bromus tectorum* has the greatest effect on wheat yield during the fall and very early spring (October through April) (Rydrych, 1974). Because *WSMV* impacts wheat after this critical period of weed competition, it may not affect its ability to suppress *B. tectorum*.

It has been suggested that increasing crop density is most useful as a weed management tool in conditions that are favourable to weeds (Mohler, 2001), represented by high N environments in our study. Our results support this assertion, showing that at high N fertilisation rates, adequate wheat seeding densities and uniform stand establishment are critical to suppress *B. tectorum* growth and spread, while crop density is less important to *B. tectorum* growth at low N rates. At low N rates, *B. tectorum* biomass was similar in parts of the field with poor stand establishment compared with areas of high wheat density. Conversely, the increase in the per-capita ability of wheat to suppress *B. tectorum* at the highest N level appeared to be driven by increases in *B. tectorum* biomass in areas with poor stand establishment. Furthermore, high wheat densities at the highest N level reduced weed biomass to levels similar to low N environments.

While we found little evidence that N and *WSMV* interact to alter crop-weed competition, the results do not exclude the possibility of interactions between the two pests, so additional research is needed before extending these results to other cropping systems. First, we did not quantify the severity of *WSMV* infection in affected wheat plants, and it is possible that infections were not severe enough to cause a reduction in competitive ability. Further, although *WSMV* presence did not impact *B. tectorum* performance consistently, the two pests may interact through other mechanisms that deserve further consideration. For example, the wheat curl mite and *WSMV* both require alternate hosts during periods when wheat is not present in the field (Christian & Willis, 1993). Volunteer wheat present in the fallow period contributes to the spread of *WSMV* by providing habitat for the virus and its vector; it is suspected that winter annual grassy weeds like *B. tectorum* play a similar role (Duffus, 1971; Wisler & Norris, 2005). In fact, in a recent field survey of *WSMV* in agricultural weeds, *B. tectorum* was the most commonly infected weed of the species sampled (Ito *et al.*, 2012). Understanding whether the presence of *B. tectorum* and other winter annual grassy weeds contribute to local *WSMV* epidemics is another critical step in investigating the interaction between these pests.

Conclusions

Interactive effects of nutrient availability and diseases may impact crop competitive ability and weed performance in agroecosystems. Previous work has established that wheat is more susceptible than *B. tectorum* to *WSMV* infection (Ito *et al.*, 2012) and that crop vigour is reduced by *WSMV* due to physiological changes (Brakke *et al.*, 1988; Price *et al.*, 2010). Our results add to previous knowledge, suggesting that reduced crop vigour due to *WSMV* infection may not alter its ability to suppress *B. tectorum*. We observed that wheat suppresses *B. tectorum* effectively even with relatively high rates of incidence of wheat infection. These results should be interpreted cautiously, as the incidence rates we observed were relatively low, and our findings do not exclude the possibility of other detrimental interactions between the two pests.

Acknowledgements

This work was supported by the United States Department of Agriculture Crops at Risk Program and the Montana Noxious Weed Trust Fund. We appreciate the efforts of three anonymous reviewers and their useful comments and suggestions for improving this manuscript.

References

- AGRIOS GN (2005) *Plant Pathology*. Elsevier Academic Press, Burlington, MA, USA, pp. 251–262.
- ALEXANDER HM & HOLT RD (1998) The interaction between plant competition and disease. *Perspectives in Plant Ecology, Evolution and Systematics* **1**, 206–220.
- ATKINSON TG & GRANT MN (1967) An evaluation of streak mosaic losses in winter wheat. *Phytopathology* **57**, 188–192.
- BLACKSHAW RE (1994) Rotation affects downy brome (*Bromus tectorum*) in winter wheat (*Triticum aestivum*). *Weed Technology* **8**, 728–732.
- BLACKSHAW RE, BRANDT RN, JANZEN HH, ENTZ T, GRANT CA & DERKSEN DA (2003) Differential response of weed species to added nitrogen. *Weed Science* **51**, 532–539.
- BRASSEY SM, WHITE JL, SAMSON RG & JOSHI J (1988) Effect of Wheat Streak Mosaic Virus infection on total DNA and chloroplast ribosomal RNA in wheat leaves. *Journal of Phytopathology* **123**, 156–164.
- BURROWS M, FRANC G, RUSH CM *et al.* (2009) Occurrence of viruses in wheat in the Great Plains region, 2008. Online. Plant Health Progress. doi:10.1094/PHP-2009-0706-01-RS. Available at: <http://www.apsnet.org/publications/apsnetfeatures/Pages/wheatviruses.aspx> (last accessed 7 February 2013).
- CHRISTIAN ML & WILLIS WG (1993) Survival of Wheat Streak Mosaic Virus in grass hosts in Kansas from wheat harvest to fall wheat emergence. *Plant Disease* **77**, 239–242.
- DORDAS C (2008) Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Agronomy for Sustainable Development* **28**, 33–46.
- DUFFUS JE (1971) Role of weeds in incidence of virus diseases. *Annual Review of Phytopathology* **9**, 319–340.
- ITO D (2011) *Evaluation of susceptibility to Wheat Streak Mosaic Virus among small grains and alternative hosts in the Great Plains*. MS thesis, Montana State University, Bozeman, MT, USA.
- ITO D, MILLER Z, MENALLED FD, MOFFET M & BURROWS M (2012) Relative susceptibility among alternative hosts prevalent in the Great Plains to Wheat Streak Mosaic Virus. *Plant Disease* **96**, 1185–1192.
- JACOBSON J, JACKSON G & JONES C (2003) Fertilizer guidelines for Montana crops, EB 161. Montana State University Extension Service. Available at: <http://animalrangeextension.montana.edu/articles/forage/fertilizer/eb161.pdf> (last accessed 7 February 2013).
- LIEBMAN M (2001) Weed management: a need for ecological approaches. In: *Ecological Management of Agricultural Weeds*. (eds M LIEBMAN, C MOHLER & C STAYER), 1–30. Cambridge University Press, Cambridge, UK.
- LIEBMAN M & MOHLER CL (2001) Weeds and the soil environment. In: *Ecological Management of Agricultural Weeds*. (eds M LIEBMAN, C MOHLER & C STAYER), 210–250. Cambridge University Press, Cambridge, UK.
- LOUDA SM, KEELER KH & HOLT RD (1990) Herbivore influences on plant performance and competitive interactions. In: *Perspectives on Plant Competition*. (eds J GRACE & D TILMAN), 413–444. Academic Press, Inc, San Diego, CA, USA.
- MALHI SS, JOHNSTON AM, SCHOENAU JJ, WANG ZH & VERA CL (2006) Seasonal biomass accumulation and nutrient uptake of wheat, barley and oat on a Black Chernozem soil in Saskatchewan. *Canadian Journal of Plant Science* **86**, 1005–1014.
- MOHLER CL (2001) Enhancing the competitive ability of crops. In: *Ecological Management of Agricultural Weeds*. (eds M LIEBMAN, C MOHLER & C STAYER), 269–305. Cambridge University Press, Cambridge, UK.
- MORROW LA & STAHLMAN PW (1984) The history and distribution of downy brome (*Bromus tectorum*) in North America. *Weed Science* **32**, 2–6.
- National Research Council; Committee on Twenty-First Century Systems Agriculture (2010) *Toward Sustainable Agricultural Systems in the 21st Century*. National Academies Press, Washington, DC, USA.
- NORRIS RE (2005) Ecological bases of interactions between weeds and organisms in other pest categories. *Weed Science* **53**, 909–913.
- NRCS (2008) USDA- Natural Resource Conservation Service. Web Soil Survey. Available at: <http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm> (last accessed 7 February 2013).
- PINHEIRO JC & BATES DM (2000) *Mixed Effects Models in S and S-Plus*. Springer, New York.
- PINHEIRO J, BATES D, DEBROY S, SARKAR D & THE R DEVELOPMENT CORE TEAM (2011) nlme: Linear and Nonlinear Mixed Effects Models. R package version

- 3.1-101. Available at: <http://cran.r-project.org/web/packages/nlme/index.html> (last accessed 1 October 2012).
- PRICE JA, WORKNEH F, EVETT SR, JONES DC, ARTHUR J & RUSH CM (2010) Effects of Wheat Streak Mosaic Virus on root development and water-use efficiency of hard red winter wheat. *Plant Disease* **94**, 766–770.
- R DEVELOPMENT CORE TEAM (2008) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- RYDRYCH DJ (1974) Competition between winter wheat and downy brome. *Weed Science* **22**, 211–214.
- SCHROEDER J, THOMAS SH & MURRAY LW (2005) Impacts of crop pests on weeds and weed-crop interactions. *Weed Science* **53**, 918–922.
- SHENNAN C (2008) Biotic interactions, ecological knowledge and agriculture. *Philosophical Transactions of the Royal Society: Biological Sciences* **363**, 717–739.
- STAHLMAN PW & MILLER SD (1990) Downy brome (*Bromus tectorum*) interference and economic thresholds in winter wheat (*Triticum aestivum*). *Weed Science* **38**, 224–228.
- TREMBLAY A & RANSIJN J (2012) A suite of functions to back-fit fixed effects and forward-fit random effects, as well as other miscellaneous functions. <http://cran.r-project.org/web/packages/LMERConvenienceFunctions/index.html> (last accessed 7 February 2013).
- WILLIAMS MM & PATAKY JK (2012) Maize dwarf mosaic can reduce weed suppressive ability of sweet corn. *Weed Science* **60**, 577–583.
- WISLER GC & NORRIS RE (2005) Interactions between weeds and cultivated plants as related to management of plant pathogens. *Weed Science* **53**, 914–917.
- WRCC (2008) Western Regional Climate Center, Historical Climate Information. www.wrcc.dri.edu. (last accessed 17 June 2011).
- ZIMDAHL RL (2004) Weed-Crop Competition; A Review, 2nd edn. Blackwell Publishing, Ames, IA, USA.