Effects of Chlorpyrifos and *Lambda*- and *Gamma*-Cyhalothrin on Suppression of Aster Leafhoppers, *Macrosteles quadrilineatus* (Hemiptera: Cicadellidae) in Spring Wheat[1]

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ABSTRACT Unexpected outbreaks of the aster leafhopper, *Macrosteles quadrilineatus* Forbes (Hemiptera: Cicadellidae), in wheat in the spring of 2012 across the Northern Plains of the U.S. prompted extensive applications of insecticides to suppress their populations. Aster leafhoppers are infrequent pests of wheat in the Northern Plains early in the spring, and data on insecticide efficacy for aster leafhoppers in wheat were unavailable at the time of the outbreak. Thus, our goal was to test several insecticides commonly used against key pests in wheat for their efficacy against aster leafhoppers. We examined the effects of chlorpyrifos and *lambda*- and *gamma*-cyhalothrin in greenhouse and field. These insecticides are not currently registered for suppression of aster leafhoppers in wheat, but they are used frequently to control potato leafhoppers in alfalfa. We found that all insecticides reduced numbers of aster leafhoppers four days after application (DAA) in the field, but not seven and 14 DAA, likely due to an influx of resident aster leafhoppers present in high numbers in the field surrounding the experimental plots. We also noted lack of effect of insecticides on total yield or the grain weight. We conclude that all insecticides effectively suppressed this pest immediately following applications, but the small-plot experiment obscured efficacy beyond the initial knockdown of the populations. These conclusions were supported by greenhouse experiments, which revealed that the insecticides killed 90% of the leafhoppers up to 14 days after application. This study will provide valuable efficacy data for new research-based chemical management recommendations for the aster leafhopper in wheat.

KEY WORDS Wheat, pest outbreaks, aster yellows, crop protection

Aster leafhoppers, *Macrosteles quadrilineatus* Forbes (Hemiptera: Cicadellidae), are frequent pests of vegetables and flower gardens, but in spring of 2012 their unusually early and large flights into wheat fields caused concern in portions of the Great Plains of the U.S., including South Dakota, North Dakota, and Minnesota (Knodel 2012, McRae et al. 2012, Szczepaniec 2012). As with most leafhoppers, the greatest concern to crop protection is caused not by direct feeding damage to the plants caused by leafhoppers, but rather by the toxic

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properties of their saliva or diseases that they transmit. Aster leafhoppers are known vectors of aster yellows (Black 1941, Nielson 1968, Frost et al. 2011), a disease caused by mycoplasma-like organisms that can cause significant losses to vegetable production (Severin 1929, Beanland et al. 2005, Frost et al. 2013). While a recent report described a fairly high incidence of aster yellows disease in wheat, oat, and barley in the Upper Midwest of the U.S. (Hollingsworth et al. 2008), aster yellows is not commonly reported on wheat and aster leafhoppers are rarely considered an economically important insect pest of wheat.

Despite the apparent lack of threat of the insects to wheat, the unexpectedly high populations of aster leafhoppers in wheat raised questions whether the newly emerged spring wheat crop should be protected. Due to a lack of recent data on the impact of aster leafhoppers on wheat or pesticide efficacy data, research-based recommendations were not available. As a result, several thousand acres of wheat were sprayed with insecticides to suppress aster leafhopper numbers in South Dakota (Szczepaniec, personal observation). While infestations of this magnitude are still rare, it is important to evaluate the impact of these uncommon outbreaks and test whether insecticide applications effectively suppress the insects and protect the crop.

Aster leafhoppers do not overwinter in the Great Plains, but migrate north from southern locations in late spring. Their overwintering hosts include small grains and weedy plants (Hagel & Landis 1967). They undergo many generations in a single season and are highly polyphagous (Hagel & Landis 1967). Aster leafhoppers can be found feeding on a variety of hosts from vegetables and flowers to turf grasses. As xylem feeders, they can cause plant stress symptoms and yield loss if insects are present in very high numbers, as has been reported for rice (Way et al. 1984), but are more likely to cause plant injury indirectly by infecting the plants with aster yellows. Symptoms of aster yellows, the disease transmitted by aster leafhoppers, resemble those of Barley Yellow Dwarf Virus (BYDV). While aster yellows infection to wheat was documented previously (Chiykowski 1967) and recently confirmed in one published case using nested polymerase chain reactions (Hollingsworth et al. 2008), it is rarely reported in wheat or considered equally threatening to wheat yield compared to BYDV. Relative to wheat, incidence of aster yellows transmitted by aster leafhoppers to barley has been much more frequently reported (Chiykowski 1963, Westdal & Richardson 1972).

The goal of this study was to test if insecticides used against key pests of wheat such as aphids suppressed aster leafhopper populations in spring wheat. We evaluated the effect of applying standard doses of chlorpyrifos and *lambda-* and *gamma-*cyhalothrin on aster leafhopper numbers in replicated plots. There are environmental risk concerns for chlorpyrifos, and its registration is currently under review (EPA 2016), but it continues to be widely used in North Dakota, South Dakota, and Minnesota and elsewhere throughout the U.S. (USGS 2013). In addition, we measured the yield of wheat and grain test weight at the end of the season to assess the impact of these insecticide applications on wheat production. Due to infrequent occurrence of aster leafhoppers in spring wheat a second year of field experiments were not possible. Thus, we supplemented the field experiments with greenhouse assays using potted wheat plants. This study provides valuable information to wheat crop managers and producers, and provides data on the management of aster leafhoppers and their effect on plants.

Materials and Methods

Field experiment. The field experiments took place near Elkton, South Dakota. Spring wheat (*Triticum aestivum*, var. Forefront) was planted on 17 March 2012 at 2.54 cm (1 inch) seeding depth and 19 cm (7.5 inch) row spacing. At the time of insecticide applications, wheat had 3 to 4 tillers of growth and densities of leafhoppers exceeded 30 individuals per five sweeps taken across four rows of wheat. Experimental plots measured 3 m by 9.1 m (10 by 30 feet) with four replicates per treatment and were separated by a buffer of four rows of wheat. The experiment was a completely randomized design. Insecticide treatments were chlorpyrifos plus *gamma*-cyhalothrin (at 0.285 kg/ha and 0.005 kh/ha, respectively, Cobalt, Dow Agrochemicals, Indianapolis, IN), chlorpyrifos plus *lambda*-cyhalothrin (at 0.242 kg/ha and 0.013 kg/ha, respectively, Cobalt Advanced, Dow AgroSciences), chlorpyrifos alone (0.54 kg/ha, Lorsban Advanced, Dow AgroSciences), and *lambda*-cyhalothrin alone (0.299 kg/ha, Warrior II, Syngenta Agrochemicals, Greensboro, NC). Foliar applications occurred on 3 May 2012 using a CO_2 backpack sprayer under 20 psi pressure, flat fan nozzles (TeeJet, XR110015) at 0.5 m (20 inch) spacing, delivered at 95 L/ha. These insecticides were selected owing to their availability and widespread use by producers.

Aster leafhopper counts were taken just prior to insecticide applications and then at 4, 7, and 14 days after application (DAA). Aster leafhoppers were sampled using a standard sweep net. Each plot was sampled by swinging the net 20 times across rows while walking along rows in each plot. Twenty sweeps covered the entire plot length. Live leafhoppers captured in the net were counted by slowly opening the net and taking note of all of the aster leafhoppers jumping out or present on the sides of the sweet net. Wheat from each plot was harvested using a small plot mechanical harvester on 20 July 2012 and data on yield and weight of grain were recorded.

Average aster leafhopper density was transformed (square root) to correct for heterogeneous variances and non-normal distribution. Numbers of leafhoppers in plots prior to treatments were analyzed using one-way ANOVA (PROC GLM, insecticide treatment as a factor in the model statement) and post-treatment data were analyzed using repeated measures ANOVA (PROC MIXED command with insecticide treatments as a fixed effect and DAA as a repeated factor) (SAS 2008). Within-date differences in aster leafhopper abundance among treatments were compared as well, and where the differences were statistically significant a post-hoc means separation test was performed (Tukey's test). Yield data failed to meet assumptions of normal distribution and homogeneity of variances pre- and post-transformations and were thus compared among treatments using a non-parametric Kruskal-Wallis test (Zarr 1999). Weight of grain was analyzed using one-way ANOVA (SAS 2008).

Greenhouse experiment. The greenhouse experiment was conducted in the greenhouse complex at South Dakota State University in Brookings, SD, USA. Wheat (var. Forefront) was sown in 3.7 L (1 gal) pots with potting mix (Sunshine® Mix # 1, SunGro Horticulture Canada, Ltd., Agawam, MA, USA) and slow-release fertilizer with 14-14-16 NPK (Multicote 4™, Haifa Chemicals, Ltd., Altamonte Springs, FL, USA). Multiple seeds were sown in each pot and were thinned down to three plants following emergence and watered ad libitum.

All experimental plants were housed in mesh cages (60 × 60 squares per cm^2 mesh; MegaView Science Co., Ltd., Taichung, Taiwan). Plants were maintained in the greenhouse at 27° ± 3°C with a photoperiod of 16:8 (L:D) h. Leafhoppers used in the greenhouse experiment were collected from wheat fields and lawns using a sweep net and moved into vials using aspirators. These leafhoppers were immediately (i.e., within hours) used in toxicity experiments described below.

Ten days following germination, two individual pots with wheat plants were assigned to one of the following treatments: untreated control, chlorpyrifos plus *gamma*-cyhalothrin, chlorpyrifos plus *lambda*-cyhalothrin, and lambda-cyhalothrin alone. Insecticides were applied with a backpack sprayer at the same rates used in the field study. Following applications of insecticides pots were immediately placed in mesh cages (two pots in each cage) and following a 24 h period 10 field-collected aster leafhoppers were placed in each cage. Survival of the leafhoppers was evaluated 4, 7, and 14 DAA of the insecticides. Any dead leafhoppers were removed from the cages and freshly collected leafhoppers were supplemented at day four and seven in order to continue efficacy evaluation based on the total of 10 individuals. Mortality of aster leafhoppers exposed to insecticides was very high and augmenting cages with live leafhoppers allowed for assessment of residual toxicity of the insecticides 7 and 14 DAA. The entire experiment was conducted twice (i.e., two time blocks), and each treatment was replicated four times in the first experiment and five times in the second experiment. The experiment was a randomized complete block design.

There was no significant interaction between the time block and treatment for the two greenhouse experiments (PROC MIXED command with treatment designated as a fixed effect and block designated as a random effect) (SAS 2008) and data from the two blocks were combined for analyses. Effects of the insecticide exposure on survival of the aster leafhoppers over time in the greenhouse experiment were measured using repeated measures ANOVA (PROC MIXED command with insecticide treatments as a fixed effect and DAA as a repeated factor) (SAS 2008) and within-date comparisons among treatments were performed in the same manner as for the field data.

Results

Numbers of aster leafhoppers did not differ among plots assigned to the treatments before the insecticides were applied ($F = 0.51$; df = 4, 15; $P = 0.727$; Figure 1). Their mean densities prior to application of treatments ranged from 164 to 224 leafhoppers per 20 sweeps.

Insecticide applications overall had a significant effect on densities of leafhoppers ($F = 4.50$; df = 4, 29.4; $P = 0.006$), while the interaction between date and treatment was not significant ($F = 1.34$; df = 8, 30.4; $P = 0.263$). The effect of treatment was largely driven by a significant reduction in leafhopper numbers 4 DAA ($F = 8.51$; df = 4, 15; $P < 0.001$; Table 1). Four days after application of the insecticides the mean number of leafhoppers in the insecticide treated plots decreased by 75-100%. Chlorpyrifos combined with *gamma*- or *lambda*-cyhalothrin had the strongest effect on leafhopper numbers and reduced

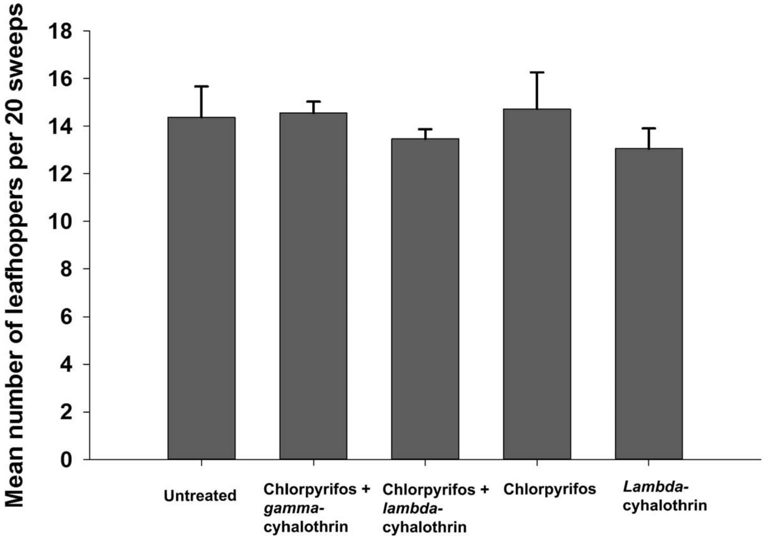

Fig. 1. Average numbers of aster leafhoppers prior to insecticide applications. Bars represent means ± SEM. There were no significant differences in average number of aster leafhoppers in plots assigned to treatments before insecticides were applied.

them to zero while untreated plots had an average of 11 aster leafhoppers per 20 sweeps (Table 1). Number of aster leafhoppers did not differ among treatments seven and 14 DAA, however ($F = 0.64$; df = 4, 15; $P = 0.643$ and $F = 1.33$; df = 4, 15; $P = 0.303$, respectively).

Table 1. Mean survival (±SEM) of the aster leafhoppers 4, 7, and 14 days after application (DAA) of insecticides in the field.

DAA	Treatment	Mean (SEM)
4	Untreated	11.0 (3.1) a
	Chlorpyrifos + *gamma*-cyhalothrin	0.0 (0.0) b
	Chlorpyrifos + *lambda*-cyhalothrin	2.0 (0.9) b
	Chlorpyrifos	2.8 (1.4) b
	Lambda-cyhalothrin	0.3 (0.3) b
7	Untreated	37.0 (11.8) a
	Chlorpyrifos + *gamma*-cyhalothrin	26.3 (4.8) a
	Chlorpyrifos + *lambda*-cyhalothrin	31.8 (14.3) a
	Chlorpyrifos	22.8 (5.4) a
	Lambda-cyhalothrin	16.3 (1.8) a
14	Untreated	46.5 (8.4) a
	Chlorpyrifos + *gamma*-cyhalothrin	32.3 (1.3) a
	Chlorpyrifos + *lambda*-cyhalothrin	34.8 (6.4) a
	Chlorpyrifos	44.8 (2.8) a
	Lambda-cyhalothrin	35.8 (5.3) a

Means with different letters are significantly different ($P < 0.05$) (Tukey's test).

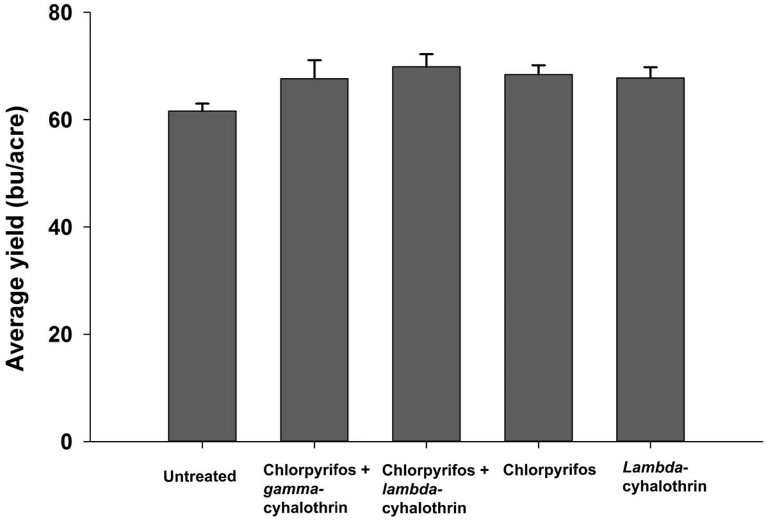

Fig. 2. Effects of insecticide treatments on yield of wheat grain. Bars represent means ± SEM. Insecticide applications had no statistically significant effect on yield of wheat.

Yield and test weight data collected at the end of the season indicate little effect of aster leafhoppers or insecticide applications on crop performance. Yield of wheat did not differ among treatments ($X^2 = 7.04$; df = 4; $P = 0.134$; Figure 2), and none of the insecticide treatments had any discernable impact on test weight of the grain compared to untreated plots ($F = 0.1$; df = 4, 15; $P = 0.98$; Untreated: 56.82 ± 1.39, Chlorpyrifos + *gamma*-cyhalothrin: 56.66 ± 1.75, Chlorpyrifos + *lambda*-cyhalothrin: 56.78 ± 1.74, Chlorpyrifos: 57.27 ± 1.7, *Lambda*-cyhalothrin: 57.14 ± 1.37).

Insecticides applied to wheat in the greenhouse had a significant effect on survival of the leafhoppers over the course of the experiment ($F = 7.81$; df = 4, 69.4; $P < 0.001$). Interaction between date and treatment was not significant ($F = 2.14$; df = 8, 70.4; $P = 0.163$). The effect of treatments on the leafhoppers was significant 4 DAA ($F = 9.18$; df = 4, 35; $P < 0.001$), 7 DAA ($F = 6.25$; df = 4, 35; $P < 0.001$), and 14 DAA ($F = 4.62$; df = 4, 35; $P = 0.005$). No live leafhoppers were noted in cages with treated plants 4 DAA, while a few insects survived on the treated plants 7 and 14 DAA (Table 2).

Discussion

This is the first report in three decades to provide an updated efficacy of currently used insecticides against aster leafhoppers in the field and greenhouse. Previously published field experiments dating back to mid-1960s and 1970 tested several chemistries that are no longer in use such as DDT, carbofuran (Furadan) (Henne 1970), and phorate (Thimet) (Richardson & Westdal 1964). These earlier studies reported that aster leafhoppers were successfully suppressed using

Table 2. Mean survival (±SEM) of the aster leafhoppers 4, 7, and 14 days after application (DAA) of insecticides in the greenhouse.

DAA	Treatment	Mean (SEM)
4	Untreated	10.0 (0.0) a
	Chlorpyrifos + *gamma*-cyhalothrin	0.0 (0.0) b
	Chlorpyrifos + *lambda*-cyhalothrin	0.0 (0.0) b
	Chlorpyrifos	0.0 (0.0) b
	Lambda-cyhalothrin	0.0 (0.0) b
7	Untreated	10.0 (0.0) a
	Chlorpyrifos + *gamma*-cyhalothrin	0.8 (0.5) b
	Chlorpyrifos + *lambda*-cyhalothrin	0.0 (0.0) b
	Chlorpyrifos	0.7 (0.4) b
	Lambda-cyhalothrin	0.0 (0.0) b
14	Untreated	10.0 (0.0) a
	Chlorpyrifos + *gamma*-cyhalothrin	0.9 (0.5) b
	Chlorpyrifos + *lambda*-cyhalothrin	1.0 (0.5) b
	Chlorpyrifos	0.9 (0.6) b
	Lambda-cyhalothrin	1.2 (0.9) b

Means with different letters are significantly different ($P < 0.05$) (Tukey's test).

malathion (Richardson & Westdal 1964), carbaryl (Henne 1970, Stevenson & Pree 1984), and permetrhin (Stevenson & Pree 1984) in vegetable crops. With the exception of our study, there are no published reports that documented the impact of these or other pesticides on aster leafhoppers in wheat.

Aster leafhoppers are not common pests of wheat in the Great Plains and their high levels of infestations in spring of 2012 were attributed to unusually warm spring months that spearheaded the robust migrations of the leafhoppers from the southern states to South Dakota. It is likely that given the widespread departures from average temperatures noted in the past several years (NOAA 2014) weather anomalies will increase in their frequency and severity. While more research on the impact of climate on insects has been conducted using forest pests, there are models that predict that agricultural pests can be affected by the changing climate as well (Porter et al. 1991, Reilly et al. 1996, Thompson et al. 2010), highlighting the need for a proactive approach to research-supported recommendations to manage them.

We report that aster leafhoppers were initially (4 DAA) controlled by all of the insecticides applied in wheat. This was previously reported in vegetable crops, which can suffer significant losses in production owing to infestation of aster leafhoppers and the pathogen they transmit (Henne 1970, Burkness et al. 1999). It is important to note that immediately following applications of the insecticides in our experiments even the untreated plots had a significantly lower number of aster leafhoppers. This may have been caused by the inherent mobility of the pest and small area of the study, which resulted in exposure of the aster leafhoppers moving across plots to insecticides and a net reduction of their numbers across all treatments. The greenhouse experiments confirm that all of the tested insecticides provide an effective initial knockdown of the populations of these insects and support the notion that sporadic contact with wheat treated with the insecticides contributed to mortality of aster leafhoppers in untreated plots.

Suppression of the leafhoppers was short-lived, however. By one week following applications of the insecticides, aster leafhopper numbers increased again and did not appear to be affected by any of the treatments. The high mobility of these insects may have contributed to this pattern. Moreover, the size of the plots (10 by 30 feet) likely obscured the impact of the insecticide applications on abundance of aster leafhoppers. The insecticide-treated plots in our experiment were surrounded by roughly 160 acres of untreated spring wheat and the highly mobile aster leafhoppers were readily moving between the untreated field and the experimental plots. While the leafhoppers may have still been deterred from the insecticide-treated plots owing to the residual toxicity of the insecticides, the constant influx of naïve aster leafhoppers not exposed to the insecticides from the surrounding wheat areas may have affected the relative abundance of the insects in our experimental plots. Nonetheless, an increasing trend in aster leafhopper numbers and lack of differences between treatments imply that control of these pests may not be feasible on small-scale and a substantially larger area may need to be treated to control this insect. Our greenhouse experiment, moreover, supports the notion that all of the tested insecticides kill aster leafhoppers and plants treated with these insecticides remain toxic to the pests for a relative long period of time. Thus, these experiments support our conclusions that influx of transient leafhoppers from the areas surrounding our experimental field plots explained the seeming loss of suppression of aster leafhoppers one and two weeks following applications of the insecticides. One other published report found that pyrethroid-based insecticides and organophosphates tested in greenhouse experiments were also highly toxic to aster leafhoppers (Stevenson & Pree 1984), thus corroborating our findings.

Negative effects of high numbers of aster leafhoppers on yield have been noted before in rice (Way et al. 1984), and yield of wheat in all of the insecticide-treated plots tended to be greater than yield of wheat in the untreated plots in our experiment. This was not a statistically significant effect, however. Aster leafhopper numbers and populations of other insects and mites in these plots were not followed beyond the 14-day experiment, and the insecticide applications had a transient impact on numbers of aster leafhoppers. It is thus not possible to evaluate how subsequent pest populations that may have colonized the crop shortly after we concluded the experiment affected crop performance. It is more likely that despite the apparent outbreak of the aster leafhoppers their impact on plant performance was insignificant.

We conclude that all of the insecticides we examined had the potential to provide effective control of aster leafhoppers based on the greenhouse data, but they did not have a lasting and profound impact on aster leafhoppers in the field. They also did not result in significant changes in the yield of wheat or quality of grain. We did not test the aster leafhoppers for presence of the mycoplasma-like organisms that cause the aster yellows and we did not assay the plants for the disease either. However, lack of observable incidence of disease in spring wheat implies that the indirect impact of these insects on plant fitness was low as well.

Predicting the impact of erratic and uncommon insect and mite pests is a difficult task. Recent unusual climactic patterns that often bring about rare pest infestations seem to suggest that this is a challenge that we will be facing with increasing frequency. Documenting the impact of these pests on crop performance and testing management strategies will improve our ability to make research-based recommendations or at least informed parallels between similar pests or pest guilds.

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