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Insecticide Applications have Minor Effects on Switchgrass Biomass Yield

Claudio Gratton,* Michael Casler, Russell Groves, and Tania N. Kim

ABSTRACT

Large-scale production of switchgrass (*Panicum virgatum* L.) as a bioenergy crop will depend on producing abundant yields without significant loss to insects. Despite the fact that crop losses to insect pests are observed in virtually every crop grown, we know little of how insects affect switchgrass biomass yield. We performed two multi-year experiments in South-Central Wisconsin where we measured Hiawatha switchgrass biomass responses to insecticides targeting aboveground and belowground insects. Experiment 1 was repeated for 3 yr in one location and Exp. 2 was repeated for 2 yr at a separate location. In Exp. 2, we also manipulated N levels to increase crop yields and potentially offset biomass loss by herbivores. We expect similar management practices to be used by farmers to reduce insect populations and increase crop yields as biomass crops become widely adopted. In the first year of Exp. 1, we found approximately 6.8% increase in yield in insecticide-treated switchgrass but the effect was not significant in subsequent years, likely due to increased variance in crop yields across plots. In Exp. 2, N application resulted in a doubling of switchgrass yield (4.65 Mg ha^{-1} compared to 9.18 Mg ha^{-1} in unfertilized vs. fertilized plots, respectively), but insecticide treatments had no effect in both years. We conclude that if insect herbivory on switchgrass is occurring aboveground or belowground, this negative impact is small relative to other sources of variation in yield and it will not likely be a source of management concern for growers in the near term.

In order for biomass crops to have a measurable benefit for national energy needs, they must be productive. Maize (*Zea mays* L.) cropping systems used for ethanol or cellulosic feedstocks, currently the most common biomass crop in North America, are managed intensively as high-input monocultures (Perlack et al., 2005). Fertilizer applications, cultivar selection, and agronomic practices such as early planting and weed management have ensured that biomass yield has increased steadily over time. Dedicated biomass crops such as *Miscanthus* × *giganteus* Greef and Deuter ex Hodkinson and Renvoize (a sterile hybrid) and switchgrass will be similarly expected to produce high biomass if they are to compete with corn as a biomass feedstock (Bouton, 2008).

However, planting of large tracts of continuous monocultures of a dedicated biomass crop as a way to meet production goals and market demands can make crops more susceptible to pest outbreaks (Root, 1973; Koricheva et al., 2000). Intensified production practices have been implicated in the buildup of pathogens and insect pests (Andow, 1991) with resulting increases in crop losses and insecticide applications to mitigate the effects of pests (Meehan et al., 2011). Pests that are resistant to insecticide can quickly exploit and sweep through a field of single crop genotypes (Gould, 1991). Moreover, natural enemies of the pests, such as predatory or parasitic arthropods, are often at a disadvantage in highly disturbed and expansive tracts of annual crops (Tscharntke et al., 2007). Predators must colonize crop fields from areas outside of the target crop, often from other source habitats that are in scarce supply in agricultural landscapes with intensified production (Griffiths et al., 2008). The consequence is a decoupling of pests and their natural enemies that can result in pest outbreaks (Segoli and Rosenheim, 2012).

Plants native to North America should be better at resisting pest damage and reducing yield loss than annual (non-native) plants (Agrawal and Kotanen, 2003). An expectation in utilizing native plants, such as warm season prairie grasses, for biomass production is that their long history of association with organisms in the native communities may offer greater resistance to disease and pest outbreaks (Macfadyen et al., 2011). If insect herbivory is a significant source of reduced fitness, then plant species or genotypes tolerant or resistant to herbivory

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C. Gratton, and R. Groves, Dep. of Entomology, University of Wisconin, 1630 Linden Drive, Madison, WI 53706; C. Gratton, M. Casler, and T.N. Kim, Great Lakes Bioenergy Research Center, Wisconsin Energy Institute, 1552 University Avenue, Madison, WI 53726; and M. Casler USDA Dairy Forage Research Center, University of Wisconsin–Madison, 1925 Linden Drive, Madison, WI 53706. *Corresponding author (cgratton@wisc.edu).

should be present in natural populations that mitigate biomass (yield) loss. Yet, there is recent evidence that native or naturalized plants currently under development as dedicated biomass crops are also susceptible to insect herbivory with potential losses of yield. For example, Prasifka et al. (2011) found several species of stem-boring Lepidoptera in stands of switchgrass but the prevalence was low with only 3% of plants infested with no expected effect on biomass yield. Similarly, *Miscanthus* infested with southwestern corn borer, *Diatraea grandiosella*, had yields as much as 30% lower compared to uninfested controls (Prasifka et al., 2012). Despite the fact that it is likely that current grass-based bioenergy crops will be consumed by native herbivores, there are few estimates of biomass yield loss beyond the scale of individual plants.

As dedicated perennial biomass crops become more widespread and markets for their use develop, pressure to increase yield will also increase. Fertility management (N application) and pest management (insecticide applications) are two practices that grass growers might increasingly use to manage perennial grasses (Heaton et al., 2004). Similarly, domesticated grasses such as corn, wheat (*Triticum aestivum* L.), and rice (*Oryza sativa* L.) are all subject to serious insect pests that are suppressed by insecticide use. If insect herbivory decreases plant production, then we would expect that insecticide applications could offset such biomass losses.

We tested the hypothesis that using broad-spectrum insecticide applications (targeting both aboveground and belowground insect fauna) in pure stands of switchgrass would increase crop yield. Increasing hectarage of dedicated bioenergy crops on the landscape and more intensified management could change crop management in the future (Perlack et al., 2005). Moreover, despite the potential for insect herbivory in switchgrass (Prasifka et al., 2011; Burd et al., 2012), there are no good estimates of yield loss from insects in this perennial crop. To test this question, we applied foliar insecticides targeting a broad range of phytophagous insects including chewing insect pests, sap-feeding insects as well as root and stem feeding insects. We also examined whether the effect of insecticide applications on yield was modified by the application of N fertilizer to grasses. In general we expected yields to increase in response to both insecticide and N applications (Gruner et al., 2008). We also expected an interaction where increasing N application might reduce the beneficial effects of insecticide application through increased compensatory plant growth. This experiment would lend information currently lacking on whether insect pest control in native grasses will be important in efforts to generate productive biomass feedstocks from perennial grasses.

METHODS

To examine whether insect pest management influences switchgrass yields we performed two multi-year experiments in two locations in replicated plots of switchgrass monocultures at the University of Wisconsin Arlington Agricultural Research Station (Columbia County, Wisconsin) in South-Central Wisconsin. The soil type is Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudoll) with <1% slope.

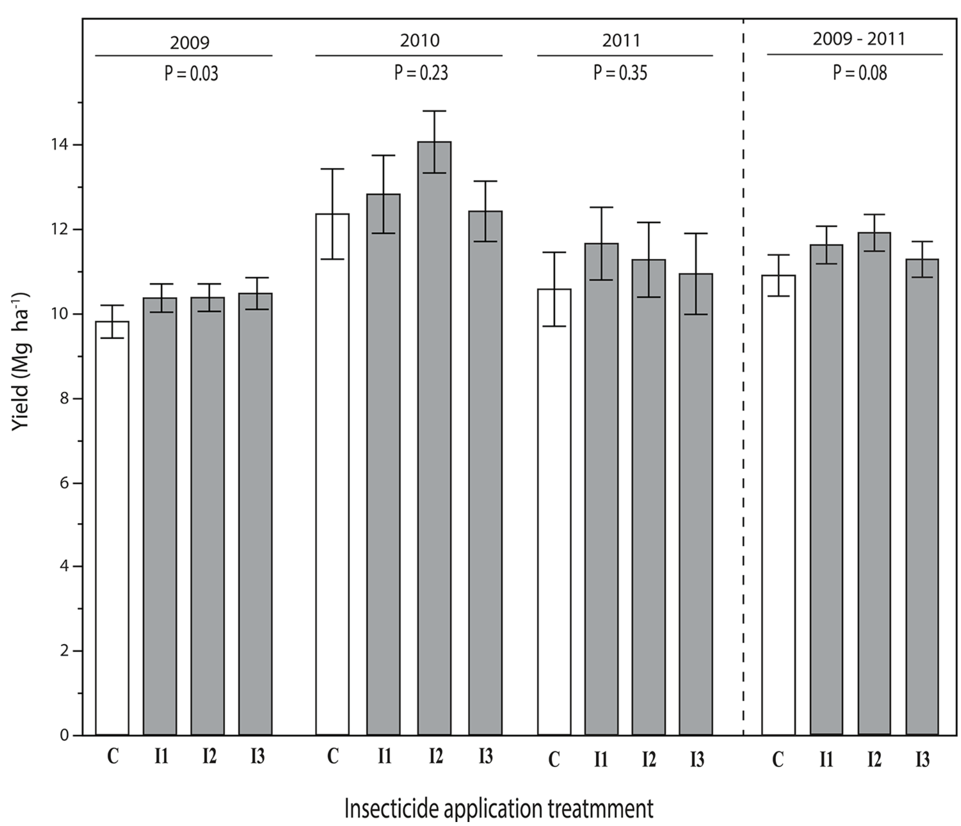

Fig. 1. Switchgrass yield (Mg dry weight ha^{-1}) in control (C) and insecticide treated plots (I1 targeted belowground insects; I2 targeted aboveground insects; and I3 targeted both belowground and aboveground insects, Table 1) at University of Wisconsin Arlington Agricultural Research Station in 2009, 2010, 2011 and the average of 2009 to 2011. Error bars represent ± 1 SE.

Experiment 1. In the first experiment, conducted in 2009 to 2011, we selected a pure mature stand of upland-type switchgrass (cultivar Hiawatha) established in 2006 (50 by 30 m). The field was split into 96 small plots (0.9 by 1.4 m) arranged in 12 rows and eight columns with 0.9-m switchgrass borders between the plots on all sides. All plots received fertilizer applications of 110 kg N ha^{-1} in early May 2009 in the form of ammonium nitrate, NH_4NO_3 (330 kg of NH_4NO_3 ha^{-1}). The experimental design was a randomized complete block with 24 blocks of four treatments (three insecticide treatments (I1-I3) and 1 untreated control (C), Table 1). To reduce soil-dwelling organisms only (I1), three foliar- insecticides were applied to plants: oxamyl (Vydate C-LV, DuPont Crop Protection, Wilmington, DE), fipronil (Regent 4SC, BASF Crop Protection, Research Triangle Park, Durham, NC), and thiamethoxam (Platinum 75SG, Syngenta Crop Protection, Greensboro, NC) which move into the soil profile and provide season-long, systemic protection against soil-dwelling organisms. To reduce foliar-feeding insects only (I2), two foliar insecticides with shorter residual activity were applied to plants: thiamethoxam (Actara 25WG, Syngenta Crop Protection, Greensboro, NC) and bifenthrin (Brigade 2EC, FMC Agricultural Products, Philadelphia, PA). To provide season-long insect control both aboveground and belowground (I3), a combination of all five active ingredients were mixed together and applied to plants. Control plots (C) only received water and all plots had a 0.9 by 1.4 m guard plot flanking the treated sections on all sides to minimize inter-plot interference. Insecticide treatments were applied 25 May, 3 June, and 5 June in 2009, 2010, and 2011, respectively in each year of the study to a short (<45 cm) and rapidly expanding switchgrass canopy. In each year of the experiment, insecticide treatments were applied to the same experimental plots so as not to confound treatments in case of inter-year carryover effects of insecticides. Application conditions were similar across years and all insecticides were applied using a hand-held, CO_2 pressurized backpack sprayer with a four nozzle boom operating at 30 psi delivering approximately 46 L ha^{-1} through four extended range, flat-fan nozzle tips (XR 8004-VS, Tee-Jet Technologies, Wheaton, IL) at 5.6 km h^{-1} applied as directed sprays to the switchgrass canopy. Application volumes of all foliar treatments were designed to exceed 45 L ha^{-1} to ensure complete canopy coverage as well as direct soil contact. Taken together, these insecticide combinations were selected to achieve long-lasting, residual control of insects in excess of 60 d during the main growth phase of switchgrass in late May and early June.

Table 1. Insecticide treatments imposed for the control of arthropod pests in the switchgrass plots located at the Arlington Agricultural Agricultural Research Station, Arlington, WI, 2009 to 2011.

Treatment	Product	Active ingredient	Rate
C	Untreated control	–	–
I1	Vydate CL-V	oxamyl	0.58 L ha^{-1}
	Regent 4SC	fipronil	0.07 L ha^{-1}
	Platinum 75SC	thiamethoxam	65.1 g ha^{-1}
I2	Actara 25WG	thiamethoxam	63.15 g ha^{-1}
	Brigade 2EC	bifenthrin	0.08 L ha^{-1}
I3	Vydate CL-V	oxamyl	0.58 L ha^{-1}
	Regent 4SC	fipronil	0.07 L ha^{-1}
	Platinum 75SC	thiamethoxam	65.1 g ha^{-1}
	Brigade 2EC	bifenthrin	0.08 L ha^{-1}
	Actara 25WG	thiamethoxam	63.15 g ha^{-1}

The insecticides used are expected to have long-lasting and broad-spectrum effects on arthropods in the switchgrass plots. Combinations of thiamethoxam and oxamyl are regarded as highly water soluble (4.1 and 280 g L^{-1}, respectively at 25°C) and will move into actively growing plant roots in 5 to 7 d post-application to affect belowground organisms and for systemic uptake. Conversely, the water solubility of the active ingredient fipronil is pH dependent, but is regarded as far less soluble in water (0.0019 g L^{-1} at pH 5.5, 20°C). Thiamethoxam is classified as a nicotinic acetylcholine receptor (nAChR) agonist with existing registrations targeting a broad range of soil and foliar insect pests. Although less soluble than either thiamethoxam or oxamyl, fipronil is regarded as systemically mobile once in the soil and will move acropetally through the vascular system of plants to growing points. This active ingredient is regarded as highly-effective against a very broad range of insect pests including both foliar and root-feeding insects similar to oxamyl. The active ingredient bifenthrin is a highly lipophillic, synthetic pyrethroid insecticide also with a very broad range of activity against many types of foliar- and sap-feeding insects. The synthetic pyrethroid class of chemicals, however, has a very short window of residual activity with many registered crop and non-crop uses. These compounds are broadly characterized as having a wide spectrum of activity often with acute contact and oral neurotoxicity to arthropods (Tomlin, 2006).

At the end of the season in mid- or late September, switchgrass plots were harvested with a flail-type harvester (1 m wide), approximately 1 to 2 wk before seed ripening in 2009to 2011. We estimated plot-level biomass yield from the estimate of total plot fresh biomass. These values were adjusted to a dry-matter basis (Mg dry matter ha^{-1}) using a grab sample of 300 to 500 g that was dried at 55°C for 5 d.

Switchgrass yield (Mg ha^{-1}) was analyzed using an ANOVA with insecticide treatment as a fixed effect and block and plot position in the field (columns) as random effects. Yield was log-transformed to meet the normality assumption of ANOVA. Data were analyzed separately for each year, and also combined into a single analysis with the addition of year and insecticide treatment ´ year as additional fixed factors and a plot (treatment) random effect to account for repeated measures of plots across years. A pre-planned contrast between control (no-insecticide) and all insecticide treatments combined was performed. All analyses were performed in JMP 10.0 (SAS Institute, 2012).

Experiment 2. A second experiment was conducted at a separate location at the University of Wisconsin Arlington Agricultural Research Station in 2010 and 2011. A 3-yr-old field of pure upland-type switchgrass (cultivar Hiawatha) was divided into 192 plots in a manner similar to Exp. 1 (24 rows and eight columns of 0.9 by 1.4-m plots). The experimental design was a randomized complete block with 16 blocks. Blocks were split into a split-plot arrangement with N-fertilization treatments as the whole-plot factor and insecticide treatments as the subplot factor. Insecticide treatments were applied in a manner identical to Exp. 1. Fertilization treatments were 0, 50, or 100 kg N ha^{-1} applied once in early May in the form of NH_4NO_3 (0, 150, 300 kg of NH_4NO_3 ha^{-1} respectively). In the second year of the study, the same treatments were applied to the same plots as in the first year. Switchgrass plots were harvested with a flail-type harvester in mid- or late September, approximately 1 to 2 wk before seed ripening in 2010–2011, and biomass yields were calculated as described in Exp. 1. Switchgrass yield (Mg ha^{-1}, log-transformed) was analyzed as in Exp. 1, as a mixed effects ANOVA but with a factorial design that included insecticide, fertilizer, and their interaction, as fixed effects.

RESULTS

Experiment 1. Switchgrass biomass in the first year of the experiment was 6.8% higher in the insecticide-treated plots (10.25 Mg ha^{-1}, control vs. insecticide contrast, $P = 0.03$) than in the controls (9.59 Mg ha^{-1}, Fig. 1, Table 2). In the two subsequent years of the experiment, however, there was no statistical difference in yield between control and insecticide treated plots (Fig. 1, Table 2) which averaged 12.23 and 10.28 Mg ha^{-1} in 2010 and 1011, respectively, across all treatments. A combined analysis of the data over the 3-yr course of the experiment showed only a marginal effect of insecticide treatment ($P =$ 0.08) that was associated with an overall increase in switchgrass yield of 8.2% over the untreated controls (Fig. 1).

Experiment 2. Switchgrass biomass was not affected by insecticide treatment in any of the years (Table 3). In contrast, nitrogen fertilization had a consistent and positive effect that more than doubled yield over the 2-yr experiment (Table 3). Unfertilized plots averaged 4.65 Mg ha^{-1} while fertilized plots averaged 9.18 Mg ha^{-1} and 10.21 Mg ha^{-1} for the 50 and 100 kg N ha^{-1} rates, respectively. The difference in switchgrass yield was significant between unfertilized and fertilized plots in each year of the study (average +109% increase, Fig. 2). Increasing the N application rate from 50 to 100 kg ha^{-1} NH_4NO_3 only increased switchgrass yield by 8%, and the effect was significant in 2010 but not in 2011 (Fig. 2).

DISCUSSION

Recent work has highlighted the possibility that insect herbivory of biomass crops has the potential to reduce biomass yield (Prasifka et al., 2012). Previous studies have found that removal of herbivores with insecticides in mixed grasslands resulted in a 13% average gain in aboveground biomass, yet the response across individual studies is often mixed (Coupe et al., 2009; Blue et al., 2011) and insecticides might have plant growth regulatory effects in addition to insecticidal properties (McKenna and Wolf, 1990). In this study, the trend of increased yield in insecticide treated plots was consistently observed across years (Fig. 1 and 2); however, in only 1 of the 5 site-year combinations was biomass statistically greater when treated with insecticides (6.8% yield gain over control).

Table 2. Summary of ANOVA for switchgrass yield (Mg ha^{-1}, log_{10} transformed) at the University of Wisconsin's Arlington Agricultural Research Station, Arlington, WI, for Exp. 1. Insecticide treatments target aboveground insects (I1), belowground insects (I2), both (I3), and a control. Nitrogen fertilization treatments were 0, 50, or 100 kg N ha^{-1}. Analyses are for 2009 to 2011 data combined (repeated measures analysis), or for each year of the study separately.

	2009–2011			2009			2010			2011		
Source	df†	F	P	df	F	P	df	F	P	df	F	P
Insecticide treatment	3, 85	1.406	0.25	3, 69	1.601	0.20	3, 69	1.399	0.25	3, 69	0.538	0.66
Control vs. insecticides‡	1, 85	3.120	0.08	1, 69	4.744	**0.03**§	1, 69	1.466	0.23	1, 69	0.899	0.35
Year	2, 184	12.391	**<0.0001**									
Year × insecticide treatment	6, 184	0.486	0.82									

† Numerator, denominator degrees of freedom.
‡ Pre-planned contrast between control and all insecticide treatments combined.
§ Values in bold represent significant *P* values ($\alpha = 0.05$).

Table 3. Summary of ANOVA for switchgrass yield (Mg ha^{-1}, log_{10} transformed) at the University of Wisconsin's Arlington Agricultural Research Station, Arlington, WI, for Exp. 2. Insecticide treatments target aboveground insects (I1), belowground insects (I2), both (I3), and a control. Nitrogen fertilization treatments were 0, 50, or 100 kg N ha^{-1}. Analyses are for 2010–2011 data combined, or for each year of the study separately.

	2010–2011			2010			2011		
Source	df†	F	P	df	F	P	df	F	P
Insecticide treatment	3, 173.6	0.038	0.99	3, 165	0.293	0.83	3, 157.6	0.176	0.91
Control vs. insecticides‡	1, 172.4	0.068	0.79	1, 165	0.372	0.54	1, 157.5	0.005	0.94
Fertilization	2, 173.7	208.006	**<0.0001**§	2, 165	209.715	**<0.0001**	2, 158.1	93.804	**<0.0001**
Insecticide × fertilization	6, 173.6	0.528	0.79	6, 165	0.650	0.69	6, 157.6	0.294	0.94
Year	1, 184.5	5.994	**0.02**						
Year × insecticide	3, 184.5	0.464	0.71						
Year × fertilization	2, 184.6	1.937	0.15						

† Numerator, denominator degrees of freedom.
‡ Pre-planned contrast between control and all insecticide treatments combined.
§ Values in bold represent significant *P* values ($\alpha = 0.05$).

Although we did not quantify herbivore pressure in this study, several mechanisms outlined below can explain the lack of difference between control and insecticide-treated plots. Deciphering mechanisms would require further investigation.

One reason for the weak insecticide effects is that herbivore pressure in switchgrass at this site is low. In another study conducted at the Arlington Agricultural Research Station in 2014 (Kim, unpublished data, 2014), damage to switchgrass in control, unmanipulated plots was low (mean leaf damage = 4.3%) compared to prairie plants in neighboring plots (mean = 8.6%) and sentinel (i.e., palatable) plants potted within switchgrass (mean = 28%). Most of the herbivores collected on plants via vacuum sampling were grasshoppers (52% of captured individuals, order Orthoptera), followed by leaf- and planthoppers (31%, order Hemiptera), aphids and thrips (11%, orders Hemiptera and Thysanoptera, respectively), and leaf beetles and caterpillars (6%, orders Coleoptera and Lepidoptera, respectively). Grasses are known to be highly resistant to insect damage relying on structural defenses such as tough leaves rich in lignin and silica, or tolerant of damage through compensatory growth (Nabity et al., 2012). These plant responses could reduce likelihood of herbivore attack on plants or reduce the magnitude of herbivore effects on yield (Tscharntke and Greiler, 1995).

Another potential explanation for weak insecticide effects could be because the insecticide treatments were not effective at reducing insect densities. Although conducting insect counts could confirm this, the suite of chemicals used in this experiment has been widely shown to be effective at reducing aboveground and belowground feeding arthropods by 40 to 80% including chewers, stem-borers, and sap feeders (Nauen et al., 1998; Potter and Held, 2002; Teicher et al., 2003; Coupe et al., 2009). These feeding guilds represent the most common herbivores known to damage switchgrass or depress yields in grass-dominated prairies (Tscharntke and Greiler, 1995; Holguin et al., 2010; Kim, unpublished data, 2014). Moreover, several of the insecticides used are known to have prolonged periods of activity, ensuring efficacy against insect herbivores well into the growing season (Nauen et al., 1998; Potter and Held, 2002; Teicher et al., 2003). The experimental design used in this study also involved re-application of the same insecticides to the same plots for either 2 or 3 yr. The systemically active insecticides (e.g., fipronil), are known to have some inter-year carryover effects in perennial plants (Wright and Cone, 1999) and could have continued to depress insects in subsequent years of the study. Thus, even if insect populations were not significantly depressed in the first year, the cumulative insecticide effect could have increased over time, particularly for soil-dwelling herbivores.

Next, variability of yield estimates increases over the course of the study in both experiments (Fig. 1 and 2) indicating that other factors such as abiotic conditions and time of harvest

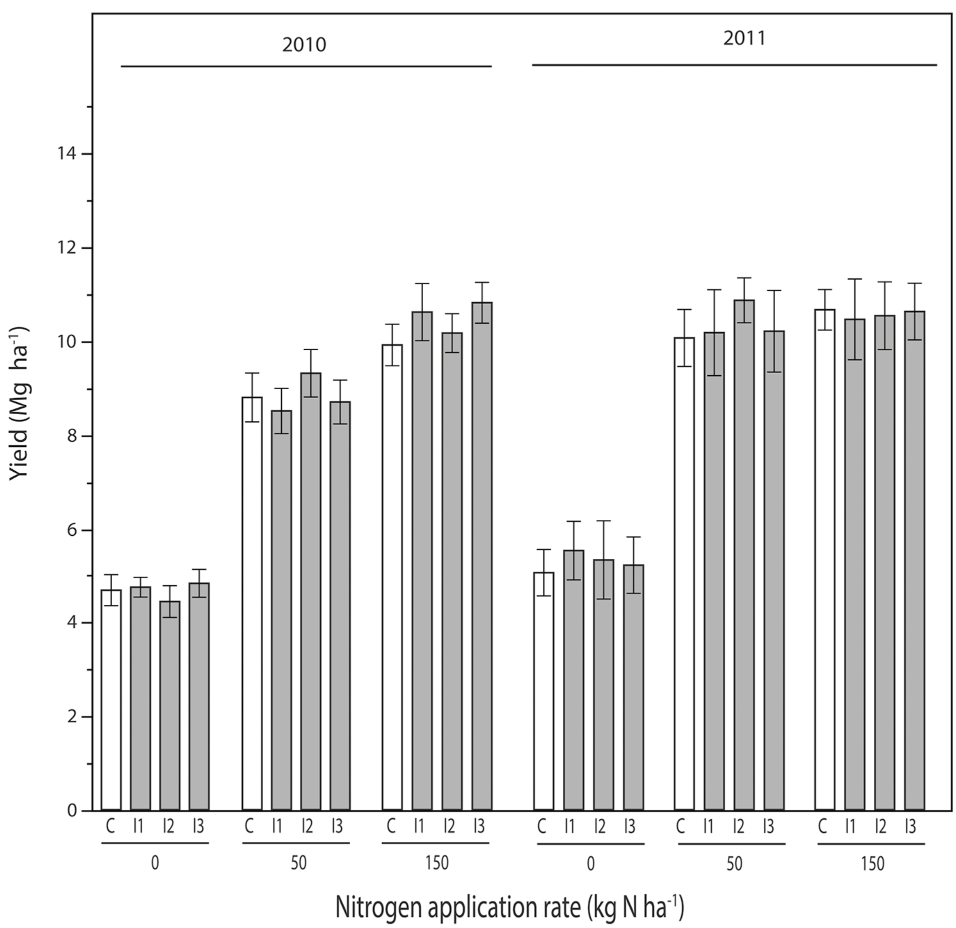

Fig. 2. Switchgrass yield (Mg dry weight ha^{-1}) in control (C) and insecticide treated plots (I1 targeted blowground insects; I2 targeted aboveground insects; and I3 targeted both belowground and aboveground insects, Table 1) crossed by N fertilization rates (0, 50, 100 kg N ha^{-1}) at University of Wisconsin Arlington Agricultural Research Station in 2010 and 2011. Error bars represent ± 1 SE.

might have larger effects on yield compared to insecticide application. For example, spatial location was significant at influencing yield (results not shown) suggesting that heterogeneity in growing conditions across the field might have larger effects compared to insect damage. Additionally, time of harvest may have introduced variability in switchgrass yield. Over the course of the study, switchgrass was harvested progressively later in the year with drier plants at the time of harvest in 2011 compared to earlier years (data not shown). As plants dry and senescence, there is an increased likelihood of biomass loss through windblown attrition which may have introduced variability in yield estimates. Because yield can be influenced by a variety of other unmeasured factors (including abiotic conditions and local management), these other sources of variability in switchgrass yield will make it difficult for growers to observe yield losses due to minor factors such as insects (e.g., <10% total yield).

The largest response of switchgrass biomass was from N fertilization treatments. The addition of 50 kg N ha^{-1} consistently doubled switchgrass biomass in each year of the study, but 100 kg N ha^{-1} rates only marginally increased biomass. Switchgrass response to N fertilization is known to be dependent on the soil type, landscape position, and differences among ecoregions (Boyer et al., 2013; Pedroso et al., 2013). The soils on which switchgrass was grown in these experiments are highly fertile, potentially limiting the response to N additions. Fertilization of switchgrass on so-called marginal lands (highly sloped and of low fertility) in southwestern Wisconsin exhibited an increase in yield fertilization rates of up to 100 kg N ha^{-1}, but not higher (Hoagland et al., 2013). Consistent with other studies, we found that fertilization with N relieves nutrient limitation, but this response is not necessarily linear (Lemus et al., 2008; Haque et al., 2009; Aravindhakshan et al., 2011). At this site, insect herbivory is suspected to be low, and fertilization did not modify the relative effect of insecticides on switchgrass yield. This implies that bottom-up effects dominate the dynamics of switchgrass rather than top-down effects by consumers.

CONCLUSIONS

Insect herbivory of biomass crops is generally low and widespread damage has not been observed in other studies (Prasifka et al., 2011). In this study, we did not observe a strong positive effect of insecticides on switchgrass yield, perhaps due to other sources of variation in crop yield. The abundance of herbivores in the experimental plots was not evaluated leaving unexplored mechanisms that may underlie the yield responses and the direct role that insects play in this particular system. Nevertheless, the multi-year, multi-location design of this study and the repeated application of insecticides (as may be practiced by grass farmers in the future) suggests that at least in southern Wisconsin, when grown on rich mollisols, herbivore effects in pure stands of Hiawatha switchgrass is generally low with only minor consequences for yield. From an agronomic perspective, grower application of insecticides to switchgrass to increase crop yield is currently unlikely to result in significant yield gains. However, regional differences in insect communities and local effects of soil and other growing conditions may create combinations where herbivory may be relevant in places other than southern Wisconsin. In addition, as cropping patterns change in response to increased demands for biomass crops like switchgrass (Perlack and Stokes, 2011), increased hectarage in more diverse locations, and more intensified cultivation practices may change the susceptibility of these native plants to insect herbivores.

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