

Bioenergy Crops *Miscanthus* × *giganteus* and *Panicum virgatum* Reduce Growth and Survivorship of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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ABSTRACT Large-scale cultivation of plants used as biofuels is likely to alter the ecological interactions of current agricultural crops and their insect pests in a myriad of ways. Recent evidence suggests many contemporary maize pests will be able to use potential biofuel crops such as switchgrass, *Panicum virgatum* L., and miscanthus as hosts. To determine how suitable these biofuels are to the maize, *Zea mays* L., pest and generalist graminivore, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), we examined host plant preference and larval performance on foliage grown for commercial biofuel production. Larvae fed leaf tissue from both field- and greenhouse-grown switchgrass and miscanthus were monitored for survival, development, and food use relative to field-grown maize. Survivorship on biofuel crops was high on greenhouse-grown leaf tissue but severely reduced for field-grown switchgrass, and no larvae survived on field-grown miscanthus. Larvae fed field-grown tissue had larger head capsules yet achieved lower pupal weights because the increased toughness of the leaf tissue prevented the assimilation of nitrogen. Given that larvae overwhelmingly preferred maize to other biofuel crop species and that survival and performance were dramatically reduced on biofuel crop species, it is likely that biofuel crops, as grown for field cultivation, will suffer reduced damage from maize pests such as *S. frugiperda* because of reduced suitability.

KEY WORDS fall armyworm, biofuels, leaf toughness, herbivory

Large-scale production of alternative (nonpetroleum) fuel sources such as plant-based biofuels is predicted to offset greenhouse gas emissions and reduce the demand for petroleum-based energy (Milliken et al. 2007). Cultivating these biofuel crops on abandoned or degraded lands may be the most carbon-neutral method (Fargione et al. 2008); however, the introduction of new monocultures adjacent to existing crops poses challenges to the ecology of agricultural landscapes. Increased plant-based biofuel production likely will alter the existing entomofauna and may exacerbate reductions already occurring in insect biocontrol (and other ecosystem) services for other crops (Landis et al. 2008). Because many of these biofuel crops are new to large-scale cultivation, it is unknown what interactions between current insect pests and these crops will arise and how these interactions will alter costs associated with production of plant-based biofuels or current agriculture.

Biofuel crops selected for cultivation require low inputs, thereby reducing costs associated with nutri-

ent management and pest control. *Miscanthus* × *giganteus* (Greef and Deuter ex Hodkinson and Renvoize; hereafter miscanthus) is a sterile (triploid) nonnative, perennial (rhizomatous) grass known for high yields, low fertilizer requirement, and broad tolerance of many soils and climates (Clifton-Brown et al. 2008). Switchgrass (*Panicum virgatum* L.) is a native perennial grass that responds well to fertilizer, is widely adapted to the climate of the central United States, and is currently cultivated as a forage crop (Parrish and Fike 2005). Both grasses suffer from minimal insect herbivory; miscanthus had no confirmed pests during the active growing of 1-yr-old field plots in England (Semere and Slater 2007), and switchgrass may only be susceptible to outbreaks of grasshoppers, aphids, and thrips depending on the cultivar and location (Parrish and Fike 2009).

Other observations identified the potential for maize, *Zea mays* L., pests to consume and develop on both miscanthus and switchgrass. When pots of miscanthus were planted adjacent to rows of maize, western corn rootworms (*Diabrotica virgifera virgifera* LeConte) laid eggs within the soil and completed development to adulthood when reared in the laboratory (Spencer and Raghu 2009); however, feeding on miscanthus reduced emergence and adult dry weight relative to insects feeding on maize. After observing fall armyworms in miscanthus field plots,

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Prasifka et al. (2009) conducted greenhouse trials that confirmed the ability of this species to complete development on both miscanthus and switchgrass. These results suggest biofuel crops may be susceptible to maize pests; however, how suitable these biofuels are as host plants for maize pests, especially under field conditions, is not yet determined.

The purpose of this study was to determine how the fall armyworm, a maize pest and generalist graminivore, performs on miscanthus and switchgrass as grown for biofuel production. Previous studies of insect survival and development on miscanthus and switchgrass examined development within greenhouse settings; however, field conditions dramatically alter the structure and nutrient availability of foliar tissue (e.g., Bazazz 1973). Therefore we compared the performance of fall armyworm on field-grown biofuels with performance on its typical host, maize.

Materials and Methods

Insect and Plant Material. Insect preference and performance experiments were conducted on field-grown miscanthus, switchgrass, and maize 'Dekalb DKC-61-72'. Miscanthus was grown in 4–5-yr-old plots located adjacent to the Soybean Free Air Concentration Enrichment (SoyFACE) facility at the University of Illinois, Urbana, IL (40° 02' N, 88° 14' W, 228 m above sea level; <http://www.soyface.uiuc.edu>). Switchgrass and refuge maize (maize lacking insect resistance traits) were grown in first-year plots at the Energy Farm facility (Energy Biosciences Institute) at the University of Illinois, Urbana-Champaign, IL. Field maize plots received an early-season application of the synthetic pyrethroid tefluthrin (Force 3G; 120 ml/300 m row), and seeds had an initial treatment of the systemic neonicotinoid insecticide clothianidin (Poncho 250; 0.25 mg/[AI] per seed), but no other pesticides throughout the season. To compare field results to previous greenhouse data (Prasifka et al. 2009), greenhouse-grown switchgrass and miscanthus plants also were used in insect trials. Switchgrass and miscanthus transplants were grown in soilless medium LCI mix (SunGro Horticulture, Vancouver, BC, Canada) in 4-liter pots, watered as needed, and fertilized weekly with a 15–5–15 ratio of N–P–K. Plants were grown at 25 ± 2°C with supplemental lighting (photoperiod of 14:10 [L:D] h).

For feeding trials, four to 10 first fully expanded, undamaged leaves were cut daily from field- or greenhouse-grown plants. Leaves were kept on ice during transport to laboratory and subsequently cut into pieces of equal area (≈800 mm²). All leaves of each host type were combined for one batch of host tissue per day. Leaf pieces were briefly (<30 min) stored in cups of water to prevent desiccation before placement in feeding trials or subsampling for elemental and chemical analyses.

All experiments used maize strain *S. frugiperda* larvae from newly hatched eggs purchased from French Agricultural Research (Lamberton, MN). Insect performance and preference tests were conducted in an

environmental growth chamber (26 ± 2°C and a photoperiod of 14:10 [L:D] h).

Performance Experiment. To measure survivorship of *S. frugiperda* on biofuel crops, larvae were reared to adult stage on tissue from plants grown in the field as well as in the greenhouse. Newly hatched larvae were placed individually in 30-ml cups (SOLO Cup Company, Urbana, IL) with fresh leaf material (collected on the same day and prepared as described above) and allowed to feed. To offset potential mortality and to ensure enough replicates by the end of the experiment, larvae that died early in the experiment were replaced with newly hatched individuals for the first 3 d. Leaf tissue was replaced and larval survivorship and instar were recorded daily.

To determine whether larvae differentially consumed or assimilated leaf tissue into body mass depending on plant species or growing conditions, a subset of developing fourth-instar larvae were weighed daily. Because insects were part of the ongoing development trial, starvation before and after consumption of test material to avoid carryover of undigested leaf material and frass was not possible. Therefore, consumption and frass produced were calculated each day for several days to account for carryover of leaf material and frass. Leaf tissue of known area was placed within a container with a preweighed fourth instar. Remaining tissue and frass were collected, dried, and weighed daily. Area and fresh weight also were measured for a subset of tissue fed to insects each day to calculate specific leaf area (SLA in square centimeters per gram) and percentage of water content before drying material. The amount of dry weight consumed was calculated as ([initial area of leaf tissue/SLA] – remaining tissue dry weight). Leaf area was calculated by photographing each leaf sample, setting the scale of the image to a known length, and then converting pixel number within the masked leaf image to area (ImageJ; Abramoff et al. 2004). Larval head capsule diameter was measured by photographing head capsules, setting the scale of the image to a known length, and converting the number of pixels across each head capsule into mm (ImageJ; Abramoff et al. 2004).

To determine the conversion efficiency of plant nutrients into insect mass, C, N, and soluble protein were measured for consumed tissue and resulting frass. For total C and N content per unit dry mass (and C:N), leaf tissue was dried at ≈100°C, ground to a fine powder, and analyzed using an Elemental Combustion System (model 4010, Costech Analytical Technologies, Valencia, CA). Soluble protein was assessed using a bicinchoninic acid protein assay (Pierce Chemical, Rockford, IL).

Preference Experiment. To test which tissues were preferred among field-grown plants, 10 newly hatched larvae were placed in the center of a petri dish ($n = 6$) lined with damp filter paper and leaf pieces of equal area (≈800 mm²) from all three species. After 24 h, the location of each insect was recorded on maize or nonmaize hosts. This experiment was conducted twice with new dishes, larvae, and leaf tissue.

Table 1. Mean (\pm SE) survival and development parameters of *S. frugiperda* on host plants selected for biofuel production

Effect	Field			Greenhouse		Species, P value	Location, P value
	<i>Zea mays</i>	<i>Panicum virgatum</i>	<i>Miscanthus</i> \times <i>giganteus</i>	<i>Panicum virgatum</i>	<i>Miscanthus</i> \times <i>giganteus</i>		
N	33	69	68	58	59		
% survival to sixth instar	61	22***	0***	59	42		
Pupation	58	20***	0***	57	41		
Adult	55	17***	0***	57	41		
Developmental time (d)							
Sixth instar	12.6 (\pm 0.2)	19.1 (\pm 0.8)***	NA ^a	13.5 (\pm 0.3)	14.8 (\pm 0.3)**	<0.0001	<0.0001
Pupation	17.9 (\pm 0.5)	25.1 (\pm 0.9)***	NA	18.5 (\pm 0.3)	20.3 (\pm 0.4)**	<0.0001	<0.0001
Adult	27.2 (\pm 0.7)	34.5 (\pm 0.8)***	NA	27.7 (\pm 0.4)	29.4 (\pm 0.4)*	<0.0001	<0.0001
Sixth instar duration	5.4 (\pm 0.4)	6.5 (\pm 0.6)*	NA	5.1 (\pm 0.1)	5.4 (\pm 0.1)	0.032	0.0005
Time to adult range	23–34	30–39	NA	24–33	26–35		
Time to adult median	27	34.5	NA	27	29		
Pupal wt (g)	0.184 (\pm 0.005)	0.184 (\pm 0.007)	NA	0.199 (\pm 0.004)	0.196 (\pm 0.004)	0.87	0.041
Eggs produced	944 (\pm 56)	1314	NA	1385 (\pm 130)	1019 (\pm 135)		
Sixth instar head capsule (mm)	3.31 (\pm 0.06)	3.55 (\pm 0.11)	NA	3.22 (\pm 0.07)	3.42 (\pm 0.07)	0.036	0.013

P values for main effects (species and location) from the two-way ANOVA are given. Significant deviation from maize for each treatment combination is indicated by *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

^a NA, not applicable.

Plant Factors Affecting Use by *Spodoptera*. To characterize plant traits that may influence insect preference and performance, we quantified leaf thickness and toughness directly. Because chlorophyll is the dominant leaf pigment and correlates with leaf nitrogen content in Poaceae (Suwa and Maherali 2008), we also quantified chlorophyll content via an indirect measure. Thickness was measured using an electronic caliper (Thermo Fisher Scientific, Waltham, MA). Toughness of field and greenhouse leaves was measured using a leaf penetrometer (see Sanson et al. 2001) where the force required to punch a rod of known diameter through a leaf of known thickness is recorded. Three measurements of force (top, middle, and bottom) were averaged per leaf and corrected for rod diameter yielding punch force: punch force = force to punch (g) \times 0.01/rod diameter \times π . Chlorophyll content was estimated with a Chlorophyll SPAD meter (Minolta Corp., Osaka, Japan), which calculates the optical density difference between two wavelengths (650 and 940 nm). Three measurements (top, middle, and bottom) of chlorophyll content were made per leaf and averaged for one leaf value.

Statistical Analyses. The unit of replication in this study was the individual larva and only surviving larvae were included in the analysis of developmental parameters. Time to stage transition (sixth instar, pupa, and adult), eggs produced, head capsule size, tissue consumption, larval mass gain, and frass production were compared with a two-way analysis of variance (ANOVA), with location (greenhouse or field) and species (miscanthus, switchgrass, and maize) as fixed effects (Proc Mixed 9.1, SAS Institute, Cary, NC). When the main effects were significant, post hoc comparisons of each treatment to maize were made using the Dunnett's test. Approximate digestibility (AD) was analyzed as described but with ingested tissue as a covariate (Proc GLM 9.1, SAS Institute). Plant traits, chemical assays, and elemental composition of tissue types were analyzed by ANOVA as described above.

The number of larvae choosing to feed on maize was compared with the pooled number choosing miscanthus and switchgrass (numbers were combined for the latter two crops and among the 12 replicates to avoid zero values). Once data were pooled, a test for independence of choice between the two experiments was performed and because there was no significant difference between experiments, ($\chi^2 = 2.32, P = 0.13$), the data for the two experiments were combined and tested for random choice between maize or miscanthus plus switchgrass (Proc Freq 9.1, SAS Institute). Survivorship on different tissue types was analyzed by location (greenhouse- versus field-grown) and species by using the survival distribution function in JMP (SAS Institute).

Results

Fall armyworm development and assimilation of host plant nutrients were impaired on switchgrass and miscanthus compared with maize (Tables 1 and 2). Survivorship to adult differed between locations ($\chi^2 = 30.04, df = 1, P < 0.0001$) and among species ($\chi^2 = 31.99, df = 2, P < 0.0001$) and was drastically reduced on switchgrass (17%) compared with survivorship on greenhouse-grown plant tissue (57%). No larvae survived on field-grown miscanthus; however, greenhouse-grown miscanthus tissue allowed for intermediate survivorship (41%). Larvae consuming field-grown tissues (maize and switchgrass) developed into smaller pupae than did larvae fed on greenhouse tissue (switchgrass and miscanthus). Larvae fed any field-grown tissue also developed larger head capsules than those fed any greenhouse-grown tissue (Table 1; Fig. 1).

Larvae fed maize grew faster during the fourth instar and assimilated more N ($\approx 80\%$) than larvae fed any other host plant (Table 2). This was likely because maize tissue also contained the highest N levels and required the least amount of force to penetrate relative to all other host plants (Table 3). Larvae also

Table 2. Mean (\pm SE) performance parameters for fourth-instar *S. frugiperda* feeding on host plants selected for biofuel production

Effect ^a	Field			Greenhouse		Species, P value	Location, P value
	<i>Zea mays</i>	<i>P. virgatum</i>	<i>M. × giganteus</i>	<i>P. virgatum</i>	<i>M. × giganteus</i>		
Ingested (g DW)	0.0244 (\pm 0.0035)	0.0226 (\pm 0.0034)	NA ^b	0.0257 (\pm 0.0018)	0.0178 (\pm 0.0023)	0.062	0.361
Frass (g DW)	0.0109 (\pm 0.0019)	0.0075 (\pm 0.0017)	NA	0.0131 (\pm 0.0011)	0.0102 (\pm 0.0011)	0.077	0.004
Wt gain (g FW)	0.0293 (\pm 0.0046)	0.0088 (\pm 0.0016)***	NA	0.0217 (\pm 0.0027)	0.0136 (\pm 0.0011)***	<0.0001	0.005
RGR (FW)	1.85 (\pm 0.32)	0.63 (\pm 0.10)***	NA	0.82 (\pm 0.09)**	0.79 (\pm 0.07)***	<0.0001	0.518
AD ^c (DW)	57.0 (\pm 5.1)	67.4 (\pm 5.1)	NA	49.9 (\pm 5.0)	49.3 (\pm 4.2)		
% C frass	45.40 (\pm 0.13)	44.16 (\pm 0.41)***	NA	42.27 (\pm 0.30)*	46.36 (\pm 0.21)	<0.0001	<0.0001
% N frass	0.77 (\pm 0.08)	0.55 (\pm 0.05)	NA	0.82 (\pm 0.08)	0.97 (\pm 0.10)	0.076	0.022
C:N frass	61.3 (\pm 4.9)	81.7 (\pm 7.9)	NA	58.0 (\pm 6.4)	51.7 (\pm 5.2)	0.069	0.012

P values for main effects (species and location) from the two-way ANOVA are given. Significant deviation from maize for each treatment combination is indicated by *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

^a Calculations for each effect were made using dry weight (DW) or fresh wt (FW) in grams.

^b NA, not applicable.

^c AD, approximate digestibility.

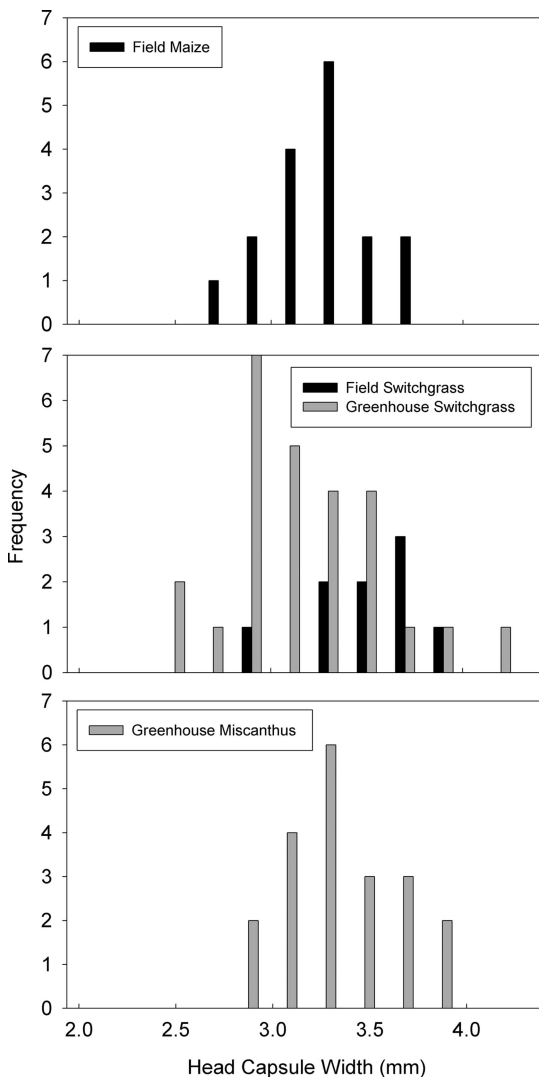


Fig. 1. Head capsule distribution for sixth instars of *S. frugiperda* fed different plant tissues.

preferred maize to nonmaize food choices ($\chi^2 = 48.7$, $P < 0.0001$), with 91% of neonates found on maize in preference tests. Maize assimilated greater N relative to all tissue types yet field-grown miscanthus and switchgrass required more force to penetrate and had reduced SLA relative to maize (Table 3).

Discussion

The fall armyworm, a generalist gramnivore, grows poorly on host plant species grown for biofuel production because leaf traits of field-grown plants reduce larval N assimilation and growth rates. Larvae failed to survive on leaves of field-grown miscanthus and displayed delayed development on field-grown switchgrass. Development rates on greenhouse-grown miscanthus and switchgrass, however, were comparable with those on field-grown maize and in our study exceeded rates reported in other studies of fall armyworm growth on greenhouse-grown miscanthus and switchgrass (see Prasifka et al. 2009). This result confirms the potential for fall armyworm to consume switchgrass and miscanthus but only under optimal growth conditions (such as those experienced in the greenhouse). Enhancing growth of field-grown miscanthus and switchgrass with N or reducing toughness via breeding out less desirable traits for biofuel production (e.g., high lignin content) will probably enhance the suitability of these crops to herbivores.

Greenhouse conditions altered the suitability of biofuel crop plants differently. As expected (Parrish and Fike 2009), switchgrass responded to fertilizer amendment by increasing N content under greenhouse conditions; however, greenhouse-grown miscanthus N content was lower than in field plants, probably because the altered spectral environment of the greenhouse reduces potential photosynthetic capacity and its related N-rich photosynthetic machinery (e.g., Rubisco, light-absorbing chlorophyll pigments; Lambers et al. 2008). When leaf toughness decreased and N content increased in switchgrass, larvae assimilated more N (and gained more weight). When both leaf toughness and N content decreased in miscanthus, larvae assimilated less N. Given that

Table 3. Mean (\pm SE) traits of host plants grown for biofuel production important to herbivores

Effect ^a	Field			Greenhouse		Species, P value	Location, P value
	<i>Z. mays</i>	<i>P. virgatum</i>	<i>M. × giganteus</i>	<i>P. virgatum</i>	<i>M. × giganteus</i>		
Water content g/g FW	78.5 (\pm 0.3)	63.1 (\pm 0.4)***	88.5 (\pm 1.3)***	77.1 (\pm 1.1)	73.8 (\pm 1.1)***	<0.0001	0.499
Leaf protein mg/g FW	39.9 (\pm 3.5)	78.0 (\pm 6.2)***	22.5 (\pm 3.3)*	31.7 (\pm 2.6)	22.1 (\pm 2.1)**	<0.0001	<0.0001
% C leaf	47.55 (\pm 0.13)	43.40 (\pm 0.22)***	47.38 (\pm 0.08)	44.11 (\pm 0.17)***	45.48 (\pm 0.24)***	<0.0001	<0.0001
% N leaf	3.46 (\pm 0.12)	1.01 (\pm 0.05)***	2.12 (\pm 0.08)***	2.29 (\pm 0.18)***	1.70 (\pm 0.06)***	<0.0001	<0.0001
C:N leaf	13.9 (\pm 0.5)	44.5 (\pm 2.3)***	22.6 (\pm 0.8)***	19.7 (\pm 1.6)*	26.8 (\pm 0.8)***	<0.0001	<0.0001
SLA (cm ² /g)	313 (\pm 4)	166 (\pm 4)***	226 (\pm 3)***	315 (\pm 19)	257 (\pm 8)***	<0.0001	<0.0001
Punch force (g/mm)	0.47 (\pm 0.02)	0.94 (\pm 0.04)***	0.88 (\pm 0.03)***	0.57 (\pm 0.04)	0.56 (\pm 0.03)	<0.0001	<0.0001
Chlorophyll proxy	45.2 (\pm 1.0)	31.1 (\pm 1.4)***	27.2 (\pm 0.7)***	26.6 (\pm 3.5)***	20.9 (\pm 2.5)***	<0.0001	<0.0001
Leaf thickness (mm)	0.21 (\pm 0.01)	0.22 (\pm 0.01)	0.18 (\pm 0.01)*	0.14 (\pm 0.00)***	0.10 (\pm 0.00)***	<0.0001	<0.0001

P values for main effects (species and location) from the two-way ANOVA are given. Significant deviation from maize for each treatment combination is indicated by *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

^a Calculations for each effect were made using fresh weight (FW) in grams.

greenhouse biofuel crop plants were similar in toughness to field-grown maize, lower assimilation of N on switchgrass ($\approx 60\%$) and miscanthus ($\approx 45\%$) relative to maize (80%) suggests another biomechanical property prevented assimilation or diluted ingested food. Furthermore, increased leaf toughness shifted the distribution of head capsule diameter toward a wider dimension (Fig. 1), yet weights were similar among treatments. This suggests increasing toughness may impart an additional energy requirement in developing mandibular muscles (see Bernays 1986) which, in combination with low N assimilation rates, exacerbates the poor suitability of switchgrass and miscanthus as food sources.

Increased leaf toughness reduces growth, increases development time, and reduces N assimilation in herbivorous insects in general (Clissold et al. 2009). Previous studies on insect consumption of miscanthus noted reductions in survivorship and development—the western corn rootworm gained less weight when fed miscanthus (Spencer and Raghu 2009) and maize-strain fall armyworm pupae were smaller and survived to adulthood at a lower rate after developing on miscanthus compared with switchgrass (Prasifka et al. 2009). These results, in addition to anecdotal evidence that fall armyworm preferred tissue in miscanthus whorls or less mature switchgrass tillers (see Prasifka et al. 2009), suggest the suitability of these biofuels is greatest when tissue toughness is lowest. Although multiple cuttings of switchgrass may provide more opportunity for infestations of less tough (younger) leaves (Fike et al. 2006), managing switchgrass and miscanthus as biofuels reduces harvest to one time point per year (to maximize aboveground biomass) and probably will reduce instances of herbivory relative to similar plots managed for forage.

Leaf toughness encompasses a combination of traits, including thickness or density of cells, amount and arrangement of cell walls, and presence of specialized silica cell types such as phytoliths (Read and Stokes 2006). Biomechanical defenses in grasses include leaf toughness and have been (loosely) negatively correlated with N content in leaves (Massey et al. 2007), possibly because the proportion of cell walls, comprising a tough, diet-diluting substance, increases

under low light and nutrient conditions relative to other cellular components (Onoda et al. 2008). However, another aspect of leaf toughness, silica content, is positively correlated with toughness yet negatively correlated with N (Massey et al. 2007). Silica concentrations of switchgrass and miscanthus vary (from 1.7 to 3.7% and from 1.0 to 2.8%, respectively), depending on field soil characteristics (M. David, unpublished data). Subsequently, suitability of these biofuels to herbivores is likely linked to soil nutrient concentrations which, in turn, alter leaf toughness. The degree to which silica content or other biomechanical properties (e.g., cell wall thickness or composition) influence herbivory and growth of these biofuel crop plants is unknown. Additional research may prove useful for guiding programs for breeding herbivore resistance in potential biofuel crops.

Miscanthus and switchgrass demonstrated reduced suitability and low susceptibility to foliage-feeding herbivores when other crops were available. This attribute enhances the productivity potential for miscanthus and switchgrass as biofuel feedstock by reducing the costs associated with pest management. Although the potential for other existing maize or grass pests (i.e., grasshoppers and aphids) to colonize miscanthus is unknown (but see Bradshaw et al. 2010), identifying the biomechanical properties underlying reduced suitability and the degree to which other maize pests assimilate biofuel crop tissues may serve as a valuable pest management tool for predicting future pest–crop interactions for plant-based biofuels.

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