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Title: Emerging wild virus of native grass bioenergy feedstock is well established in the Midwestern USA and associated with premature stand senescence

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Abstract

The North American native prairie grass *Panicum virgatum* (switchgrass) is a primary bioenergy feedstock candidate. Its widespread distribution and genetic diversity enable the possibility of developing this perennial grass for high production in a variety of conditions, including on marginal lands. A critical concern in feedstock development and deployment is the risk of novel pathogen emergence. Here we investigate the landscape-scale prevalence and epidemiology of a little-studied North American virus first detected in switchgrass and other grasses in bioenergy trials in the US Midwest. *Switchgrass mosaic virus* (SwMV, Genus *Marafivirus*, Family *Tymoviridae*) is transmitted by leafhoppers and phylogenetically sister to *Maize rayado fino virus,* a significant pathogen of maize in parts of the Americas. Our goal was to determine whether SwMV is uniquely limited to specific bioenergy trials or well-established and circulating more broadly. We used molecular diagnostics to quantify naturally occurring SwMV infection in leafhoppers and switchgrass in naturalistic stands throughout a large Midwestern landscape, and quantified leafhopper abundances and stand performance. Our analysis revealed that this apparently wild virus is well-established and widespread. Infection was present at nearly all sites, across diverse landscape contexts, with prevalences ranging as high as 33%–60%. Infection appeared to accumulate and persist in stands over time. It was associated with increases in premature stand senescence but not with reductions in stand height. Although wild viruses are believed to evolve benign relationships with their natural hosts, these data suggest that SwMV has potential to impact yield components. Viruses are frequently overlooked in crop development efforts, but represent the majority of emerging plant pathogens. For SwMV, it is imperative to quantify its impact on host performance, to identify the extent of any host resistance, and to assess any risks of virus spillover to agricultural plantings of other Poaceae species, including maize and sorghum.

Keywords

Plant virus ecology, Switchgrass mosaic virus, Tymoviridae, marafivirus, leafhopper, *Graminella, Panicum virgatum*, pathogen, bioenergy, prairie grass

Introduction

Perennial grasses are important potential feedstocks for sustainable production of cellulosic bioenergy (Lemus and Lal 2005, Somerville et al. 2010, Robertson et al. 2017). In the United States and elsewhere, a primary feedstock candidate is switchgrass (*Panicum virgatum* L.)—a genetically diverse C4 prairie grass native to North America (Sanderson et al. 1996, McLaughlin et al. 2002, McLaughlin and Adams Kszos 2005). Switchgrass is found in numerous natural habitats, from native prairies to riparian areas, across a wide geographic range and latitudinal gradient (Casler et al. 2004, Lowry et al. 2014). Switchgrass is a strong candidate for bioenergy production because it produces substantial biomass with modest crop inputs (i.e., nitrogen), can tolerate marginal lands, and provides multiple ecosystem services (Mitchell et al. 2008, Mitchell et al. 2012, Werling et al. 2014).

To develop switchgrass as a bioenergy crop, it is essential to evaluate the extent and nature of pathogen infections it may acquire in the field. Such infections could reduce switchgrass yield and quality, and potentially increase disease pressure on other Poaceae crops (Schrotenboer et al. 2011). To date, most attention has focused on identifying and controlling fungal rust infections, which are readily apparent in both lowland and upland switchgrass ecotypes (Zale et al. 2008, Hirsch et al. 2010, Kenaley et al. 2018, VanWallendael et al. 2020). However, initial data indicate that switchgrass is also susceptible to multiple pathogenic crop-infecting viruses known to damage cereals, sugarcane, and turf grasses, including *Barley and cereal yellow dwarf viruses* (B/CYDVS, Family *Luteoviridae* (Garrett et al. 2004, Schrotenboer et al. 2011), *Panicum mosaic virus* (PMV, Family *Tombusviridae*) and its synergistic dependent *Satellite Panicum mosaic virus* (SPMV) (Sill and Pickett 1957, Scholthof 1999, Stewart et al. 2015), and *Sugarcane mosaic virus* (SCMV, Family *Potyviridae*) (Agindotan et al. 2010). Some of these viruses are vectored by flying insects, including sap-feeding leafhoppers and aphids, that can spread infection locally and over long distances.

As a native prairie grass, switchgrass likely arose 2 MYA in the Pleistocene and has had a long presence in North America, where it still can be considered a 'wild' non-crop species in contrast to domesticated grasses (Parrish et al. 2012). As indicated by recent high-throughput sequencing of crop and non-crop vegetation, non-crop plants harbor a rich diversity of plant viruses that are only beginning to be explored (Roossinck et al. 2010, Min et al. 2012, Bernardo et al. 2017, Susi et al. 2017, Shates et al. 2019). Initial investigations of the switchgrass virome in Illinois (USA) identified two novel species: the tentatively named *Switchgrass mosaic-associated virus 1* (SgMaV-1, Genus *Mastrevirus*, Family *Geminiviridae*) (Agindotan et al. 2015), and *Switchgrass mosaic virus* (SwMV, Genus *Marafivirus*, Family *Tymoviridae*) (Agindotan et al.

2010, Agindotan et al. 2012), which is the focus of this study. SwMV is transmitted by the grass-feeding leafhopper *Graminella aureovittata* (Agindotan et al. 2013b), a species associated with moist prairies in the central and eastern USA (DeLong 1948).

Crop-associated viruses (henceforth 'crop viruses') that cause economic loss in crops have so far received most attention in plant virology (Wren et al. 2006, Alexander et al. 2017). Crop viruses can have significant negative effects not only on crops but also on non-crop vegetation (Malmstrom and Alexander 2016). Crop-associated BYDV, for example, can stunt switchgrass root systems (Malmstrom et al. 2017) and reduce the biomass production and integrated multi-year fitness of switchgrass plants (Alexander et al. 2017). In contrast, almost nothing is known about the effects of non-crop 'wild' viruses such as SwMV on either crop or non-crop vegetation. It has been suggested that most non-crop virus infections have little negative impact on hosts and might even be beneficial (Fraile and García-Arenal 2016, Roossinck and Bazán 2017). Among the very few wild viruses of plants that have been studied, effects on hosts were found to be slightly negative to neutral (Alexander et al. 2020) or contextually dependent (Gibbs 1980). In the case of SwMV, there is potential for damaging impact. Its nearest known relative, *Maize rayado fino virus* (MRFV), is arguably the most important viral pathogen of maize in Latin America (Gámez 1969, Rybicki 2015), raising the question of whether SwMV likewise might be pathogenic in its hosts.

We discovered SwMV in Michigan switchgrass about the same time that Agindotan et al. (2010) reported infection in bioenergy trial plots in Illinois and Wisconsin. These parallel discoveries prompted us to investigate the distribution and impact of the novel virus to better understand whether it might pose a threat to perennial grass feedstocks. We began by asking whether the elevated SwMV prevalence seen in the bioenergy trials might represent a unique situation, perhaps influenced by cultivation conditions, or whether SwMV infection was instead widespread, with these initial reports representing just the "tip-of-the-iceberg" of its distribution. Because the first SwMV detection in Michigan was in a conservation planting, not a feedstock trial, we chose to investigate the distribution of infection in established naturalistic stands throughout our region. We reasoned that if infection were found throughout these little-managed stands, it would be good indication that the virus was well established in our area and not unique to a few bioenergy trial plots. As little is known about the virus' epidemiology, we further sought to identify possible impacts of infection on stands and to assess whether local site properties or the nature of the surrounding landscape might predict its distribution. To do this, we quantified the prevalence of SwMV, the abundance of potential leafhopper vectors, and relationships between SwMV prevalence and stand conditions at sites in different

landscape contexts throughout a 37,000-km^2 area of Michigan, USA. We used a SwMV-specific molecular diagnostic to quantify SwMV prevalence in both switchgrass and the native *Graminella* leafhopper species that feed on it, including the known SwMV vector *G. aureovittata* (Agindotan et al. 2013b). Our study coincided with a severe summer drought, which we quantified at each location with a drought index.

We found that SwMV infection was widespread and present at all but one of our 15 sites. Moreover, infection prevalence was the best predictor of switchgrass senescence in the drought, suggesting that infection damaged stressed stands, perhaps by reducing their stress tolerance. An alternative explanation—that drought or poor stand growth increased infection prevalence—was not supported by statistical models. Landscape context did not predict prevalence patterns or abundance of known vectors, suggesting that virus and vector pressure is a synoptic phenomenon filtered by site properties. Taken together, these findings strongly indicate that SwMV infection is well established in our region and merits attention as a pathogen of potential virulence. More broadly, these findings highlight the need to better understand how selection of new crops influences their relationships with endemic wild viruses and the risk of emerging infectious disease.

Materials and Methods

Virus system

Switchgrass mosaic virus (SwMV) is a positive-sense single-stranded RNA virus (Family *Tymoviridae*, Genus *Marafivirus)* that is transmitted to grasses by leafhoppers (Agindotan et al. 2012, Agindotan et al. 2013b). In plant hosts, marafivirus virions are most often found in phloem and xylem tissues (Nault and Ammar 1989). In switchgrass, SwMV infection may produce straight fine white, creamy, or yellowish lines and dots in leaves, running parallel to the veins (Fig 1A, B) and similar to symptoms of MRFV in maize (Zambrano et al. 2013). However, some infections are asymptomatic (Agindotan et al. 2013b). Infection overwinters in switchgrass rhizomes and re-emerges with new tillers in the spring (Ryskamp et al., in prep); thus, prevalence values represent infections accumulated over multiple years.

Marafiviruses propagate within their insect vectors (insect hosts), as well as within the plant host, and vectors require a latent period of at least one week after virus acquisition before transmission (Nault and Ammar 1989). Tests with the leafhoppers *G. aureovittata*, *G. mohri*, and *Flexamia atlantica* (all members of

the Family *Cicadellidae,* Order *Hempitera*) found that only *G. aureovittata* (Fig. 1C) transmitted infection (Agindotan et al. 2013b). Marafiviruses are not known to be transmitted by seed (Nault and Ammar 1989) or by mechanical means in the field, although vascular puncture transmission is possible in the lab (Weiland and Edwards 2011). SwMV has been detected in several C4 Poaceae species in Illinois besides *P. virgatum,* including in the North American natives *P. amarum (*bitter panicum*), Andropogon virginicus* (broomsedge bluestem), and *Sorghastrum nutans (*Indian grass*),* and in several species of the non-native *Miscanthus* genus (Agindotan et al. 2013a). Beyond these findings, the biology of this emerging and apparently native virus remains largely undescribed.

Study approach and locations

We evaluated naturally occurring virus dynamics in naturalistic stands that were planted in the past. This approach represents Method 2 for studying plant virus effects in the field (Experimental plants with naturally occurring virus infection) with some elements of Method 1 (Natural plant populations with naturally occurring virus infection), as not all information about planting material was known and the plantings had self-propagated and spread (Malmstrom and Alexander 2016). The study examined established switchgrass-dominated communities with upland switchgrass ecotypes at 15 sites in 12 counties across Michigan's lower peninsula in the Great Lakes Region (USA) (Fig. 2). Thirteen of these stands were established in the 1990s–early 2000s for conservation purposes (*e.g.,* game bird habitat) in state game areas and on private property, and were left largely undisturbed or managed only lightly; ten of the latter were included in a related study of ecosystem service provisioning by switchgrass and prairie communities (Werling et al. 2014). In addition, we included two regularly harvested larger switchgrass plantings established in 2010 as "scale-up" sites for the US Department of Energy-supported Great Lakes Bioenergy Research Center (GLBRC). The GLBRC scale-up fields were seeded with upland *P. virgatum* cv. Cave-in-Rock, an octoploid natural-track cultivar from Illinois (Evans et al. 2015). Specific seeding records for the other fields were not available, but Cave-in-Rock was most commonly used in such plantings in Michigan during that time period. Field size ranged from 0.5–14 ha (median = 4 ha) with most fields 2–6 ha in area; the two scale-up sites were the largest (13–14 ha). Nearest-neighbor distances between points ranged from 4.4 km to 63.0 km.

Sites were chosen to represent a range of landscape contexts with differing proportions of crop and non-crop cover types. To quantify landscape context, we evaluated the distribution of the 2012 US Department of Agriculture's Cropland Data Layer (CDL) land cover types (https://nassgeodata.gmu.edu/CropScape/) within circular buffers with radii of 0.5 km (79 ha area), 1.0 km

(314 ha area) and 1.5 km (707 ha area) around each site; the 2012 CDL is a geo-referenced raster with 30-m ground resolution. All GIS work was conducted in ArcGIS versions 10.6 – 10.8 (ESRI, Redlands, CA, USA). We aggregated the land cover types represented in our region into eight primary cover groups: (i) Agricultural cover, which included four dominant crops --maize, winter wheat, alfalfa, soybeans—and lesser amounts of 19 other vegetable, fruit, and small grain crops; (ii) Grass/meadow; (iii) Developed; (iv) Forest; (v) Wetlands; (vi) Miscellaneous perennials; (vii) Barren; and (viii) Open water (see Supplemental Materials for further description). Across all sites and at the three scales, agriculture represented 32–33% of the cover in this diverse landscape; grass/meadow, 17–31%; forest, 13–17%; developed areas, 12–14%; wetlands, 10–16%; and open water, 2–4% (Fig. S-1 A–C).

Drought conditions

In 2012, the US Midwest experienced unusual dryness and drought (Rippey 2015), and the effects in Michigan were spatially heterogeneous. To quantify how much drought each study site experienced, we calculated a drought index value based on spatially-explicit estimates of the duration and weekly severity of drought conditions as published in the US Drought Monitor (https://droughtmonitor.unl.edu/) . The Drought Monitor rates moisture conditions as no drought or dryness (no drought rating), abnormally dry (D0), moderate drought (D1), severe drought (D2), extreme drought (D3), and exceptional drought (D4). For each site's GPS location (*i*), we thus calculated a Drought Index (*DI*) as

$$DI_i = \sum_{j=1}^{n} [if\ d_{i,j} \geq 0, (d_j + 1); else\ 0]$$

where j = the growing season week, and d_j = the Drought Monitor D value rating (0 – 4) for week j. Thus, weekly ratings of D0, D1, D2, D3, and D4 were valued as 1, 2, 3, 4, and 5 respectively. Weeks with no recorded drought or dryness were valued as 0. Switchgrass in this region typically sprout in early May, so we calculated *DI* for the 16 weeks from the week of May 1 through the week of August 14, when early August field measures were completed (i.e., n = 16).

Switchgrass condition and sampling for SwMV detection

To quantify relative differences in stand productivity among sites, we measured switchgrass height at 15 within-stand locations at each site in both sampling periods and calculated mean canopy values. To characterize stand condition, we quantified the degree of stand senescence by estimating the percentage of senesced switchgrass foliage (dry brown leaves) at 15 random points at each site.

For virus detection, foliar tissue was sampled from switchgrass in late August at the twelve accessible sites. At each site, we collected tissue from fifty plants sampled every 1.5 meters along two 70-m transects that were at least 20 m apart. At each transect point, we sampled the tiller closest to the point, without regard to size, condition, or symptoms. After collection, samples were transported on ice and then stored at -20°C until processed. At one site (Sw07), we were able to compare prevalence values with earlier 2010 collections from switchgrass (n = 41 plants) and big bluestem (*A. gerardii*) (n = 3 plants).

Leafhopper collection & identification

Our prior field observations suggested that *Graminella* were most abundant in our area in late summer. To confirm that seasonal distribution, we sampled leafhoppers from June – August at four sites (SW02, SW07, SW10, SW14) selected to represent diverse geographic regions. We sampled our larger network of sites twice in August, when *Graminella* numbers were greatest: In early August (August 2–8, 2012), we sampled all 15 sites, and in late August (August 23–29) we sampled 12 of the 15 sites, as SW09, SW17, and SW18 could not be accessed. All collections occurred during warm and sunny daylight hours (10 am – 4 pm); air temperature was recorded. There are multiple methods for capturing leafhoppers; we used sweep-netting because in our experience this method is the best approach in our system when fresh samples are required for virus analysis. For each collection, we captured leafhoppers from three separate transects of 50 sweeps each, spaced at 1 sweep per meter, for a total of 150 sweeps. Captured insects were killed by immersion for 10–15 minutes in a jar containing ethyl acetate, transferred into plastic bags, and stored in a cooler before long-term storage at -20°C.

We sorted leafhoppers from plant debris and other arthropods in the sweep samples with a sieve and microscope. In Illinois switchgrass stands, Agindotan et al. (2013b) found *G. aureovittata, G. mohri,* and *F. atlantica* and determined that only *G. aureovittata* transmitted SwMV. In our sweep collections, nearly all the leafhoppers were *Graminella* spp. We did not find any *F. atlantica*, and to the best of our knowledge this species has not been recorded in Michigan. We also did not find any *Dalbulus maidis* or *G. nigrifrons*, which transmit MRFV (the crop-infecting relative of SwMV) to maize. *G. aureovittata* was readily identified by its characteristic shape and orange stripes (Fig. 1C). The remaining *Graminella* were a mixture of *G. mohri,* and *G. oquaka* (Fig. 1D), which look highly similar to each other (DeLong 1948). To identify these individuals to species, we dissected a subset and evaluated the male genitalia. Part of the abdomen of each sampled individual was removed and placed in a heated 10% potassium hydroxide solution for 30 min to expose the

internal male parts, then washed in distilled water and placed in glycerin for inspection under microscope following a modified version of the method of Oman (1949) (Trębicki et al. 2010). The aedeagus was then evaluated with the DeLong (1948) key. To preserve samples for RNA extraction, further sorting was non-destructive. *G. aureovitatta was* sorted easily based on morphological characteristics alone. Because *G. mohri* and *G. oquaka* could not be distinguished without destructive analysis and then only males could be properly identified, we grouped these two sister species together as *G. oquaka/mohri*. Sorted leafhoppers were then stored at 20°C until viral RNA could be extracted. For the early August sample, data for *Graminella* are complete but counts of total leafhoppers (all taxa collected) are missing from 3 sites (SW11, SW12, SWLA). In late August, the sample from SWLA was damaged partway through analysis so that from it only counts of *G. aureovittata* are available.

Detection of SwMV

We used molecular diagnostics to detect SwMV in a subset of the leafhopper and plant tissue samples collected. In total, we tested 180 switchgrass plants for infection and 192 leafhoppers (44 *G. aureovittata* and 148 *G. mohri/oquaka*). At each sampled site, we tested 16 switchgrass individuals (every third individual from each 50-plant collection, with field locations ≥ 4.5 m apart). For leafhoppers, we tested all *G. aureovittata* collected, except for a few individuals reserved for species confirmation, because this species is known to transmit SwMV. In the early August collection, we also tested a subsample of 10 *G. mohri/oquaka* from each site. At sites where fewer than 10 individuals were collected, we tested all that were available. In late August, when *G. mohri/oquaka* were less apparent, we tested individuals from only one site (24 individuals tested of 29 collected).

RNA extraction for virus detection

From switchgrass, we extracted total RNA with the Spectrum Plant Total RNA extraction kit (Sigma-Aldrich), according to the manufacturer's protocol. 100 mg of frozen leaf tissue was homogenized for 2 minutes in the Mini-Beadbeater-16 (BioSpec Products, USA) in a 2-mL screw-cap tube containing liquid nitrogen and 1.0-mm silica-zirconium beads. After homogenization, 500 µL of lysis solution containing 5 µL of 2-mercaptoethanol was added to each tube and vortexed for 30 s. The solution was incubated at 56°C for 5 min and then centrifuged for 12 min at 15,000 RCF to pellet cellular debris. Next, the supernatant was transferred to a filtration column and centrifuged for 1 min at 15,000 RCF. To capture RNA, the flow-thru

lysate from the filtration column was mixed with 750 µL of binding solution and transferred to the binding column. Tubes were centrifuged for 1 min at 15,000 RCF. After washing, RNA was eluted from the binding column with nuclease-free water and 1 µL of RNaseOut ribonuclease inhibitor was added. RNA was stored at -80°C until further analysis.

From leafhoppers, we extracted total RNA using a modified Dellaporta method (L. Ingwell, pers. comm.) (Dellaporta 1983). For each batch of 16 leafhoppers, 10 mL of Dellaporta extraction buffer was prepared in a nuclease-free glass container from 1.0 mL of 100 Mm Tris at Ph 8.0, 1.0 mL of 500 mM EDTA, 1 1.25 mL of 500 mM NaCl, and 6.75 mL of nuclease-free water. Immediately before use, 10 µL of 2-mercaptoethanol was added to the buffer. Each leafhopper was homogenized for 10 s using the Mini-Beadbeater-16 in a 2-mL screw-cap microcentrifuge tube containing 400 µL of Dellaporta extraction buffer and 1.0-mm silica-zirconium beads (BioSpec Products). To disassociate nucleo-protein complexes, each sample was next incubated with 52.8 µL of 10% SDS solution for 10 minutes at 65°C. After incubation, 128 µL of 5 M potassium acetate solution was added to facilitate protein and DNA removal, and the samples were centrifuged at 4°C for 10 min at 15,000 RCF, resulting in a pellet. Next, 480 µL of supernatant was transferred to a new tube and centrifuged for another 10 min at 15,000 RCF at 4°C. To precipitate RNA, each sample was incubated with 240 µL of cold 100% isopropanol at -20°C for 1 hr and then centrifuged at 4°C for 20 min at 15,000 RCF. The isopropanol was removed and discarded, leaving the pellet, which was washed with 70% ice-cold ethanol and centrifuged. After the ethanol was removed, pellets were air dried for 10 min. Finally, the RNA pellets were resuspended in 80 µL of nuclease-free water with 1 µL of RNaseOut ribonuclease inhibitor (Sigma-Aldrich) and stored at -80°C.

RT-PCR amplification and Sanger-sequencing of amplicons

We used reverse-transcription (RT) to convert viral RNA from plant and insect samples to cDNA, which was then amplified with PCR. Total RNA concentrations were quantified with the Qubit Fluorometer 2.0 (Life Technologies). In reverse transcription, 1 µg of total RNA (to a maximum of 5 µL for more dilute samples) was added to a mixture containing 0.4 µL of 10 µM reverse primer (BO88-MRFV-10R: 5'-GCC CAC AGG TCT TAT GGC CGA CCT GCT ACC -3' (Agindotan et al. 2010)) and 4 µL of 10 mM dNTPs (Sigma Aldrich), previously mixed, and nuclease-free water was used to bring the total reaction volume to 12 µL. Mixtures were incubated for five minutes at 65°C and then in ice for five more minutes to promote annealing. Next, 7 µL of a master mix containing 4 µL of 5X first-strand buffer (Sigma), 2 µL of 0.1 M dithiothreitol, and 1 µL of RNaseOut ribonuclease inhibitor (Sigma) was added to each tube. Finally, each tube received 1 µL of

SuperScript II enzyme (Sigma-Aldrich) for a final RT reaction volume of 20 µL. Samples were incubated at 42°C for 50 minutes to promote DNA polymerization, and then 15 minutes at 70°C to inactivate the enzyme.

We then performed PCR on the cDNA to amplify a 635-bp region of the viral coat protein, following a modified version of the Agindotan et al. (2010) protocol. Briefly, 2 µL of diluted RT product (1 /10 dilution in nuclease-free water) was added to a 0.2-mL PCR tube containing 18 µL of master mix: 2 µL of 10X PCR Buffer, 1.2 µL of 25 mM $MgCl_2$, 1.6 µL of 10 mM dNTPs, 0.8 µL each of 10 µM reverse primer (used in RT) and forward primer (5'- GCTATTCCTGCTCCTCCTCGTGTGGTTGAAACC-3'), 0.2 µL of AmpliTaq Gold enzyme (Sigma-Aldrich), and 11.4 µL of nuclease-free water. Final reaction volume was 20 µL. RT product was diluted to limit inhibition of downstream PCR reaction. Amplification was performed using a Peltier Thermal Cycler (PTC-200, MJ Research) as follows: activation at 94°C for 10 minutes, followed by 40 cycles of denaturing (94°C, 30 s), annealing (60°C, 30 s) and extension (72°C, 45 s), with a final extension (72°C, 10 min). The PCR product was analyzed on a 1.25% ethidium bromide gel under UV light. DNA amplicons were purified with the QIAquick PCR Purification or the QIAquick Gel Purification kit (QIAGEN). Purified DNA was submitted with forward and reverse primers to the Genomics Technology Support Facility (Michigan State University, East Lansing, MI, USA) for Sanger sequencing.

Statistical analysis and ecological predictors

Statistical analysis was conducted in JMP Pro version 15 (SAS, Cary, North Carolina USA), except as noted. We used generalized regression with native distributions and model selection methods with Akaike information criterion values (AIC_c, corrected for small sample size) (Burnham and Anderson 1998, 2002) . The best distribution for each response variable was determined by comparing AIC_c values and weights for fits with appropriate choices. Calculation of a global Moran's Index for each response variable in ArcMap 10.8 did not find evidence of spatial auto-correlation (Table S-1). $N = 15$ for all models except those in which SwMV prevalence in switchgrass was the dependent variable or appeared as a predictor in at least one candidate model, for which $N = 12$. For null models, we included those with intercept only, or with only intercept and latitude or longitude. We considered the best model to be that with the lowest AIC_c value and present as competing models those for which $\Delta AIC_c \leq 2$.

We first evaluated potential predictors of two aspects of switchgrass *stand performance* in early August: (i) *mean stand height* (Weibull distribution), a measure of stand growth related to productivity, and (ii) *mean stand senescence* (mean percent dry leaves, log-normal distribution), a measure of stand condition.

For both, we evaluated several models with intercepts and single explanatory variables describing local conditions (*i.e.,* drought index, *DI*, or switchgrass infection prevalence). For stand senescence, we further considered stand height as a single predictive variable, and models with both switchgrass infection prevalence and drought index, and with both factors and their interaction.

We next evaluated models of *local and landscape factors* that might explain patterns of two key elements of the virus system: (i) *Graminella abundance* in early August (negative binomial distribution) and (ii) *SwMV prevalence in switchgrass* (exponential distribution with the single zero data value converted to 0.001). Abundances of the leafhoppers and virus prevalence both have potential to be shaped by local stand conditions as well as by landscape-level supply and the extent of landscape provisioning of biocontrol. For *Graminella* abundance, we evaluated four relevant metrics of local site conditions (drought index, field size, height of switchgrass, temperature at time of collection). For SwMV prevalence in switchgrass, we considered three site properties (drought index, field size, stand height) three measures of vector abundance (abundances of *G. aureovittata* and of all *Graminella* in early August, and total August abundance of *G. aureovittata*), and early August measures of SwMV prevalence in *G. aureovittata, G. oquaka/mohri*, and in all *Graminella*. Finally, we evaluated the influence on both response variables (*Graminella* abundance and SwMV prevalence in switchgrass) of the proportions (within 0.5 km, 1.0 km, and 1.5 km buffers around each site) of three of the eight land cover groups previously described: (i) wetlands and (ii) grass/meadows, which might provide *Graminella* habitat and SwMV reservoirs; and (iii) agricultural cover, which likely would not. We also considered the contribution of two land cover groups whose proportions in 1.5-km buffers were associated with increased biocontrol in this region: (i) forests and (ii) an additional category of herbaceous perennial habitat (Werling et al. 2011b) that includes alfalfa, shrublands, clover/wildflower, and three cover types from the grass/meadows group (other hay, fallow/idle crop, and pasture/grass). Proportions were calculated as the proportion of all land cover in that buffer.

Results

Drought and switchgrass condition

The record 2012 drought affected all 15 of our switchgrass sites (Fig. 2). Dry conditions developed earliest (week of May 29) and were most prolonged in the south-western end of our sampling network, but by late July all sites were experiencing at least moderate drought and the majority (13/15) were in severe to

extreme drought (Fig. 2). Drought index (*DI*) values ranged from 10 to 31 (median = 19) and declined with latitude (R^2 =0.567, $F_{(1,13)}$ = 17, p=0.0012). Switchgrass canopy height (the mean of 15 measures per stand) in early August varied more than two-fold among sites (63 –140 cm, Fig. S-2), while percent dry leaves ranged from 3.5% (SW15 in mid-Michigan) to 24% (SW01, SW08, southwest Michigan) (Fig. S-3). By late August, canopies were more senesced (percent dry leaves: 4 – 46.3%). Some stands had grown considerably taller, others less so (Fig. S-2, mean heights: 75–150 cm; height increases: 7%–103%), and one dry southern stand (SW08, *DI* = 23) was beginning to shrink (-1.3%).

Abundance of *Graminella* leafhoppers

The June – August time series of collections at four sites confirmed that *Graminella* abundance was greatest in late summer (Fig. 3). In June, leafhoppers were captured at all four sites, but no *Graminella* were found, and in July, *Graminella* counts were low. *Graminella* abundance peaked at three of the four sites (SW02, SW07, SW10) in early August. At the remaining site (SW14), abundance was greatest in late August.

The extensive August collections across the full network of 15 switchgrass sites yielded more than 1,218 leafhoppers in total, of which 914 individuals were *Graminella* (Fig. 4A, Table S-2). *Graminella* were found at all sites, and this genus was the dominant taxon at most, comprising 40–100% of the leafhoppers in all but two of the collections (Fig. 4B). Among the *Graminella*, the known SwMV vector *G. aureovittata* was much less abundant than its congeners *G. oquaka* and *G. mohri* (Figs. 4A, C), representing ~4.8% of total *Graminella* captured across both dates. In the total August collection, we found no *G. aureovittata* at all at three sites (SW09, SW13, SW14) where other *Graminella* (*N* = 36–151 individuals) were collected.

Graminella were most abundant in early August, in which we caught 637 individuals (Fig. 4A, Table S-1). *Graminella* were found at all sites except in one north-eastern location (SW11, Huron County). At the other 14 sites, collection counts ranged from 2–162 individuals per 150 sweeps (median = 37). Of the *Graminella* caught across all sites, 96.9% (617/637) were *G. oquaka/mohri* and just 3.1% (20/637) *G. aureovittata* (Fig. 4A, B, Table S-1). On a per-site basis, the numbers of *G. aureovittata* never exceeded those of *G. oquaka/mohri* and were generally much smaller (Fig. 4A, B). The percentage of *Graminella* that were *G. aureovittata* thus ranged from a high of 50% at SW12 where only 2 *Graminella* were caught (one of which was a *G. aureovittata)* to 0% (4 sites), with a median value of 2.3%.

In late August, leafhoppers were less abundant overall and the total number we caught, as well as the number of *Graminella*, fell at most sites (median per-site decline -28% and -31% respectively) (Fig. 4A, B).

At the 11 collection sites, *Graminella* counts ranged from 3–96 per 150 sweeps (median = 19). *Gr. aureovittata* abundance remained low but did not decline and we caught 24 individuals across 12 sites (median = 1).

Switchgrass mosaic virus (SwMV) prevalence in switchgrass and *Graminella*

Reverse-transcription (RT)-PCR tests of 180 switchgrass samples and 413 individual leafhoppers revealed that SwMV was widely distributed across our study region. The virus was detected at 14 of the 15 switchgrass sites we sampled, either in switchgrass foliage, in leafhoppers, or in both (Fig. 5A). Infection was found in switchgrass leaves at 11 of the 12 sites at which the species was sampled, with prevalence ranging 6.7% – 60%. At one site with notable infection (Sw07), we were able to compare prevalence values from 2010 and 2012 and found little change (63.4% to 60.0%) (Fig. 5B). Two of the three 2010 samples from big bluestem—a species not previously known to host SwMV—were infected as well (Fig. 5B).

Graminella leafhoppers were caught in sweeps at 14 of the 15 sites but patterns of virus detection in them were bifurcated. At sites where virus prevalence in *P. virgatum* foliage was less than ~20%, we detected little to no virus infection in the leafhoppers, except at one site (SW14) where virus was found in all *Graminella* tested (Fig. 5A). In contrast, when foliar prevalence exceeded 20%, the majority of *Graminella* tested positive (75–100%). At two of the three sites where plant data were missing (SW17, SW18), a large proportion of *Graminella* were positive for virus, suggesting that prevalence in the *P. virgatum* was likely also notable.

Ecological predictors

Best predictors of stand properties. The extent of premature stand senescence, reflecting stand condition in early August, was best predicted by SwMV prevalence, not by drought index, latitude or longitude, stand height (a measure of growth related to productivity), or multi-factor models (Table 1, Fig. 6). Stand height was not associated with SwMV prevalence or drought index in either early August (Table 2, Table S-3) or late August (data not shown).

Influence of local site factors on vector and virus prevalence. Graminella abundance was best predicted by stand height in early August (Table 2, Table S-4). SwMV prevalence in switchgrass was negatively

associated with field size and positively associated with SwMV prevalence in *G. aureovittata, G. oquaka/mohri*, or *Graminella* overall (competing models, Table 2, Table S-5).

Influence of land cover context. Land cover analysis showed the diversity of landscape contexts for the sites in this study. At the 1.5km-scale, agriculture was the largest category of land use for 7 of the 15 sites (Fig. S-4). Wetlands were dominant at three others, grasslands/meadows at two, forest at one, and the remaining two had notable developed land use nearby (Fig. S-4). However, neither *Graminella* abundance nor SwMV prevalence was predicted by proportions of any of the five land cover groups, representing possible habitat/reservoirs or sources of biocontrol, that we evaluated (Tables 1, S-4 and S-5).

Discussion

Viruses cause the majority of emerging infectious diseases in plants (Anderson et al. 2004), and these diseases are likely to only increase in importance despite efforts to control them (Nicaise 2014). At present, the leading driver of viral pathogen emergence is anthropogenic introduction of viruses to new hosts or regions, sometimes called 'pathogen pollution' (Anderson et al. 2004). Other current drivers include introduction of or increases in vector populations, altered agricultural practices, and virus evolution (Rojas and Gilbertson 2008). Deeper in time, however, virus emergence was likely driven by human domestication of plants and the rise of agriculture. Gibbs et al. (2008), for example, found evidence that agriculture drove the emergence of potyviruses and their prevalence in crops. Recent geometagenomics analysis supports this idea, finding associations of several virus groups with agricultural land use (Bernardo et al. 2017). Because switchgrass is still close to its roots as a wild prairie grass, having experienced only a few cycles of selection for forage, conservation, and bioenergy (Parrish et al. 2012), its development as a bioenergy feedstock presents unique opportunities to watch domestication in action but also raises risk of driving new viral disease emergence. Our finding that the wild marafivirus, *Switchgrass mosaic virus* (SwMV), is well-established in Mid-Western USA agro-ecological landscapes raises crucial questions about its potential impact on bioenergy feedstocks, its epidemiological drivers, and risk of spillover to other crops such as maize.

SwMV infection is widespread

As of this writing, SwMV infection has been discovered in switchgrass in bioenergy plantings in four Midwestern US States: Illinois (Agindotan et al. 2010, Agindotan et al. 2013a), Michigan (two sites in this

study), Missouri (Malmstrom, Lowry, et al., unpublished data), and Wisconsin (Agindotan et al. 2010) (Fig. S-5). Our study is the first to examine the distribution and prevalence of SwMV in more naturalistic conservation plantings across a diverse agro-ecological landscape. We found SwMV infection to be ubiquitous in these systems with its prevalence reaching 30 – 60% in a quarter of the stands (Fig. 5a) and persisting across years (Fig. 5b). These findings demonstrate that this recently identified virus is not uniquely limited to a few bioenergy plantings but rather demonstrates characteristics of an established and endemic wild virus. This conclusion is reinforced by longitudinal studies in progress that document significant virus presence in stands over time (Malmstrom et al., unpublished data). Developing understanding of SwMV ecology and epidemiology are thus important in assessing risk of significant disease emergence and impact.

In bioenergy trial plots in Illinois, Agindotan et al. (2013a) reported SwMV infection in ten different switchgrass cultivars (lowland and upland ecotypes), as well as in three other native grasses (*A. virginicus, P. amarum var. amarum, S. nutans)*, and several introduced species (*Miscanthus* spp. and *Saccharum ravennae),* indicating that this virus is a multi-host generalist, not a switchgrass specialist. Our findings expand knowledge of its host range to include *A. gerardii* (big bluestem), meaning that at least three of the four dominant species of North American tallgrass prairie (*A. gerardii, P. virgatum, S. nutans*) support SwMV infection. The fourth native dominant—*Schizachyrium scoparium*—has not yet been evaluated but may also prove to host SwMV because it belongs to the same *Saccharinae* subtribe (tribe *Andropogoneae*, subfamily *Panicoideae*) as three other hosts (*Miscanthus, Sorghastrum*, and *Saccharum).* It is possible that SwMV has been endemic in the tallgrass prairie for an extended period, but the extent of its influence requires further investigation. We speculate that at present the virus may be more common in the moist Eastern side of the tallgrass prairie region as infection has not yet been reported from virus surveys of switchgrass in drier regions, including Kansas (Malmstrom and Alexander, unpublished data) and Oklahoma (Muthukumar et al. 2009) (Fig. S-5).

Potential impact on switchgrass

The impact of wild plant viruses on their natural hosts remains poorly understood. It is increasingly suggested that wild viruses serve as commensalists or mutualists, either little perturbing or benefitting their hosts (Fraile and García-Arenal 2016, Roossinck and Bazán 2017), particularly when infections are asymptomatic or latent (Takahashi et al. 2019). In switchgrass, however, SwMV infection frequently is

symptomatic (Fig. 1). Given its ubiquity, prevalence, and phylogenetic relatedness to the maize pathogen *Maize rayado fino virus* (MRFV), SwMV merits consideration as a potential pathogen of note. To investigate effects of natural SwMV infection in the field, we examined relationships between SwMV prevalence and switchgrass height, as a proxy for stand growth, and between prevalence and the extent of stand-level senescence in early August, as a metric of growing-season condition and stress. In our area, switchgrass can remain green until hard frosts in October, so senescence in early August is ca. two months premature. We found no relationship between SwMV prevalence and stand height, suggesting that infection did not detectably limit initial canopy development (Table 2, Table S-3). Notably, SwMV prevalence was the best predictor of stand-level senescence (Table 1), with greater prevalence associated with greater senescence.

These findings suggest two possibilities about the nature of SwMV influence on switchgrass. One possibility, in keeping with the hypothesis that wild viruses confer benefits on hosts, is that SwMV serves as a mutualist that permits infected plants to better tolerate drought—a frequently posited benefit of infection (Xu et al. 2008, Westwood et al. 2013). In this conceptual model, stands with greater senescence might have greater SwMV prevalence because infected plants were favored under drought stress and persisted while uninfected individuals succumbed. However, this scenario is not the most congruent with our data. For example, it would be most consistent with a statistical model that included prevalence, drought index, and their interaction, but the prevalence-only model (with intercept) was the AIC_c-best fit (Table 1). Moreover, in this multi-stemmed perennial species, infected individuals do not vanish abruptly but rather senesce gradually, and we sampled senescing individuals. So even if senescence happened faster in uninfected plants (*i.e.,* if infection increased stress tolerance), our measure of prevalence would probably not have been much influenced. Related hypotheses that drought or poor stand growth increased infection prevalence were not supported by statistical models.

A more straight-forward explanation of the data is that SwMV is a pathogen that does not impede stand height gain but instead provokes premature senescence, perhaps exacerbated by drought. While this suggestion is at odds with the idea that wild viruses typically are not pathogenic in their natural hosts, it is supported by the frequent expression of symptoms in infected switchgrass. The extent to which premature senescence might translate to reduced bioenergy production depends on the interplay among its effects on biomass yield, nutrient resorption, and conversion efficiencies (Ong et al. 2018). While any optimization of harvest timing can be complex and specific to the conversion process (Ong et al. 2018), premature senescence shortens the growing period and is likely to reduce yield potential in all cases. Moreover, if

premature senescence extends the time between senescence and harvest date, it provides additional opportunities for dry biomass to be lost to wind or drop to the ground beyond the reach of harvesting equipment (Adler et al. 2006, Anderson et al. 2013). Overall, we conclude that SwMV appears to be an endemic virus with capacity to achieve notable prevalence and potential to reduce yield quantity or quality in switchgrass. It merits careful attention in feedstock development and raises fundamental questions about factors that might allow maintenance of virulence in wild viruses.

Ecology and epidemiology of SwMV

A broad literature documents the influence of surrounding landscapes on pest/natural enemy dynamics and implications for pest management (Landis et al. 2000, Bianchi et al. 2006, Meehan et al. 2011, Werling et al. 2011a, Werling et al. 2014, Gurr et al. 2017, Karp et al. 2018, Haan et al. 2020). In this study, however, we found local site factors to be better predictors of *Graminella* abundance or SwMV prevalence than any landscape elements reflecting habitat or reservoir opportunities, or biocontrol sources. *Graminella* abundance was predicted only by the local factor of switchgrass stand height. None of the other local factors or any of the landscape factors, each with potential to influence vector abundances, was found to be influential. Studies of leafhopper responses to local and landscape factors in other systems reveal a range of relationships that differ among species, with some likewise demonstrating no clear associations with landscape factors (e.g., Vaidya et al. 2017). In our system, *Graminella* abundance may increase with stand height because of the increased structural complexity of the vegetation or changes in microclimate; *Graminella* nymphs, for example, seem to prefer the shade within deeper canopies (E. Cole, personal observation). Alternatively, *Graminella* abundance might increase with stand height to the degree that height reflects stand productivity and carrying capacity.

SwMV prevalence in switchgrass was best predicted by the proportions of *Graminella* leafhoppers testing positive for the virus (several positive relationships), and by field size (a negative relationship), but not by *Graminella* abundance. The negative relationship with field size is counter-intuitive, and we suspect that field size is serving indirectly as a measure of time since stand establishment because the largest stands in our study were the most recently established. Since infection can overwinter in rhizomes (Ryskamp et al., in prep.) and persist for several years as evident at one of our sites, prevalence might be expected to accumulate over time. However, we could not more precisely test the influence of stand age because the specific establishment years of the older stands were unknown.

Competing models of switchgrass infection prevalence that contained leafhopper factors included prevalence in *Gr. aureovittata* (a known vector), in *Gr. oquaka/mohri* (one designated non-vector, one untested), and in all *Graminella* (Table 2). This result mirrors the identification of vector infectivity proportions as critical parameters in disease risk assessment in agriculture (e.g., Frost et al. 2013). The congruity of virus prevalence in leafhoppers and plants underscores the biological linkage between the two populations, and suggests that sampling either one can provide useful information about infection prevalence within a stand. Insects have proven to be valuable integrators of virus signals within plant communities in both vector- and predator-enabled metagenomics (Ng et al. 2011, Rosario et al. 2013, Rosario et al. 2015). It is possible that laboratories experienced with insect identification might find testing leafhoppers for virus to be simpler than working with plant samples, which requires overcoming issues with tissue toughness and biochemistry (Lacroix et al. 2016). Interestingly, both our study and Agindotan et al. (2013b) found that in switchgrass stands with notable infection (> 20%), virus was detected in a greater proportion of leafhoppers than of plants, whereas in stands with lesser levels of infection (≤ 20%) the opposite was true: we generally, but not always, detected virus less frequently in leafhoppers than in plants (low prevalence stands were not evaluated in Agindotan et al.). For disease monitoring, these results imply that detection of notable SwMV prevalence in *Graminella* indicates high likelihood of notable infection within the stand itself.

For epidemiological analysis, a key question is the degree to which virus signal detected in different *Graminella* species reflects their capability to transmit the virus (either effectively or in a limited manner), or merely reflects ingestion of virus particles. One species we sampled, *Gr. aureovittata*, was previously found to transmit SwMV while *G. mohri* was not (Agindotan et al. 2013b). The transmission efficiency of *G. oquaka* remains untested and merits attention. In our study, *G. aureovittata* comprised only 3.1% of the *Graminella* population, leading us to wonder whether the more abundant *G. oquaka/mohri* group might contribute to infection spread. We could not determine the relative proportions of *G. oquaka* and *G. mohri* in our study because the two species look highly similar and the destructive identification measures needed to distinguish them were incompatible with virus testing. We therefore recommend expanded testing of the transmission efficiencies of both species and suggest that particular attention should be paid to nymphs, which are the most efficient vectors of other marafiviruses (Nault and Ammar 1989). Alternatively, if *G. aureovittata* proves to be the primary vector, its low numbers in 2012 may have been a short-term anomality caused by the drought, as DeLong (1948) reported that this species has greater affinity for damp environments than *G. oquaka or mohri.*

The lack of detectable effects of landscape cover (0.5 – 1.5 km distances) on *Graminella* abundance and SwMV prevalence is intriguing. We considered the influence of two land covers that might harbor *Graminella* and/or Poaceae hosts of SwMV (wetlands and grass/meadow) and one (agriculture) that likely would support neither. We also evaluated the influence of two potential sources of biocontrol: forest cover and perennial herbaceous cover (Werling et al. 2011b). In contrast to the local predictors, none of these landscape metrics showed any significant relationship with *Graminella* abundance or SwMV prevalence and did not contribute to the AIC_c-best fit models. However, there was a marginal ($P = 0.068$) negative effect of forest area within a 1.5-km buffer on SwMV prevalence (but not *Graminella* abundance) worth future investigation. More generally, the lack of significant landscape signal at the scales we considered, along with the widespread finding of SwMV infection, suggests that SwMV and *Graminella* may be broadly dispersed across this landscape with site conditions serving as modulators that amplify or diminish their presence. As winged insects, leafhoppers can be widely distributed and "rain" across many vegetation types within a landscape (e.g., Keene et al. 2020). Moreover, the natural pest suppression supply generated by forests and herbaceous perennial landscapes (Werling et al. 2011a) may be ineffective at controlling leafhopper populations.

Implications for disease emergence

Our data indicate that SwMV deserves attention as a potential driver of yield or quality loss in switchgrass and a possible emergent pathogen in feedstock development. Selection of native plant material for production (domestication) may inadvertently increase virus susceptibility (Schrotenboer et al. 2011), although not always (Nygren et al. 2015). The phylogenetic relatedness of SwMV to the maize pathogen *Maize rayado fino virus* (MRFV) indicates need to consider the risk of a host jump by SwMV to maize or related crops. The factors currently limiting spread of SwMV infection to maize are not known but may reflect vector distributions. MRFV is transmitted primarily by *Dalbus maidis*, but also by the widespread *Graminella nigrifrons*, an abundant herbivore on Poaceae in the Eastern US (DeLong 1948). SwMV is transmitted by at least one *Graminella* species and potentially others. Given the capacity for RNA virus evolution, it is important to consider the possibility that widespread planting of switchgrass might create opportunities for SwMV to develop capacity for transmission by other leafhoppers, including *Gr. nigrifrons*,

and thus potentially to infect maize. Identification of resistance to SwMV and selection for it during feedstock development might reduce risk of these scenarios.

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Author Contributions

CM conceived the study and supervised it. DL and BW contributed to the design and establishment of the site network; BW arranged permission for sampling. PT and CM planned the field work. EC and PT collected leafhoppers and field tissue. PT and AK Busch identified leafhoppers. AK Busch and EC conducted the molecular bench work. AK Brown and CM conducted the spatial analysis, and DL and BW provided insight about landscape factors. CM and PB curated and wrangled the data, and CM conducted the statistical analysis. AK Busch, EC, and PT contributed to initial draft components, and CM wrote the primary draft. CM and AK Brown designed and made the figures. All authors reviewed and contributed to the submitted manuscript.

Data Availability

The data that support the findings of this study are openly available in the Dryad data repository (https://datadryad.org) at http://doi.org/10.5061/dryad.bk3j9kddv.

Table 1. Statistical models explaining the extent of canopy senescence (mean percentage dry leaves) in switchgrass stands in early August 2012 (generalized regression with a log-normal distribution). AIC_c-best model is in bold.

Models of canopy senescence (*N* = 12)	***Effect***	AIC_c	ΔAIC_c	P_{Factor}	R^2
Null models					
Intercept only		78.6	2.4	<0.0001	0
Latitude		81.6	5.4	0.410	0.034
Longitude		80.1	3.9	0.497	0.163
Site properties					
Prevalence (switchgrass)	**+**	**76.2**	**0**	**0.0052**	**0.394**
Drought index (DI)		79.0	2.8	0.091	0.192
Canopy height		80.9	4.7	0.234	0.106
Prevalence (switchgrass), drought index		79.0	2.8	0.0088,0.144	0.486
Prevalence (switchgrass), drought index, interaction		84.9	8.7	0.143, 0.112, 0.556	0.501

Table 2. **Summary of model selection statistics for AIC_c-best models (or competing best models) for four response variables considered in this study. *N* = 15 except for analyses including *Prevalence* (switchgrass), where *N* = 12. For full set of models evaluated, see Table 1 (switchgrass senescence), Table S-3 (switchgrass height), Table S-4 (*Graminella* abundance), and Table S-5 (SwMV prevalence in switchgrass).**

Response variable	Explanatory variables	*Effect*	*N*	AIC_c	Δ AIC_c	*Model P-value*	*Model* r^2
Switchgrass height	Null model - Intercept only	N/A	15	141.1	0	<0.0001	0
Switchgrass senescence	Prevalence (switchgrass)	+	12	76.2	0	0.0052	0.394
Graminella abundance - early August	Switchgrass height	+	15	141.9	0	0.0003	0.432
Prevalence(switchgrass)	Prevalence (*G. aureovitatta*) in early August	+	12	92.6	0	0.027	0.385
Prevalence(switchgrass)	Prevalence (*Graminella)* in early August	+	12	93.5	0.9	0.034	0.333
Prevalence(switchgrass)	Prevalence (*G. oquaka/mohri*) in early August	+	12	93.6	1.0	0.036	0.329
Prevalence(switchgrass)	Field size	–	12	94.6	2.0	0.020	0.271

Figure Legends

Figure 1. Organisms examined in this study. (a) *Switchgrass mosaic virus* (SwMV) infection in field-grown upland switchgrass. (b) Close-up of SwMV infection symptoms in upland switchgrass foliage. (c) Known SwMV vector *Graminella aureovittata*. (D) Congener *G. oquaka. Photos: C. Malmstrom.*

Figure 2. Growing season drought index (DI) for 2012 at network of 15 field sites in 37,000-km^2 region of southern and mid-Michigan, USA. Map shows site locations colored by severity of *DI,* derived from severity of drought conditions (none – D4) as published in the US Drought Monitor (https://droughtmonitor.unl.edu/); see text. Panel below shows drought conditions at each site during individual weeks of growing season (May 1 – August 14) and corresponding *DI.* No value: normal moisture; 1-abnormally dry (D0); 2 - moderate drought (D1); 3 - severe drought (D2); 4 - extreme drought (D3).

Figure 3. Growing season time series of *Graminella* spp. abundance in 2012 at four sites distributed across sampling region shows peak values in August. Values are number of individuals of all *Graminella* species (*G. aureovittata* and *G. oquaka/mohri*) per 150 sweeps. Gray-shaded periods represent early August sampling (A) and late August sampling (B).

Figure 4. Leafhopper abundance at 15 switchgrass field sites in early August (August A, black bar) and late August (August B, grey bar), ordered by abundance of *Graminella* spp. in early August. Values are number of individuals per 150 sweeps. (a) Total number of individuals of *Graminella* spp. captured. (b) Total number of individuals of *Graminella* spp. as a percentage of all leafhoppers caught. (c) Number of *G. aureovittata*, a known vector of SwMV. Note differences in Y-axis scale among panels. Dark asterisk, no data for August A; light asterisk, no data for August B.

Figure 5. Prevalence of naturally-occurring SwMV infection in established upland *P. virgatum* (switchgrass) stands. (A) Prevalence in early August 2012 across all sites, as detected in *Graminella* leafhoppers (light bars) and in *P. virgatum* foliage (dark bars). SwMV prevalence in *Graminella* spp. is weighted by the relative abundance of taxa. Sites are ordered by SwMV prevalence in *Graminella* (highest to lowest). '0' indicates zero prevalence in either *P. virgatum* (blue) or *Graminella* (orange). 'NG' indicates that the site was swept for leafhoppers but no *Graminella* were captured. Asterisk (*) indicates *P. virgatum* samples were not collected. (B) Comparison of SwMV prevalence in *P. virgatum* at site SW07 in 2010 and 2012, with 2010 values for prevalence in *A. gerardii* (big bluestem).

Figure 6. Prevalence of SwMV infection is best predictor of stand senescence (see Table 1).

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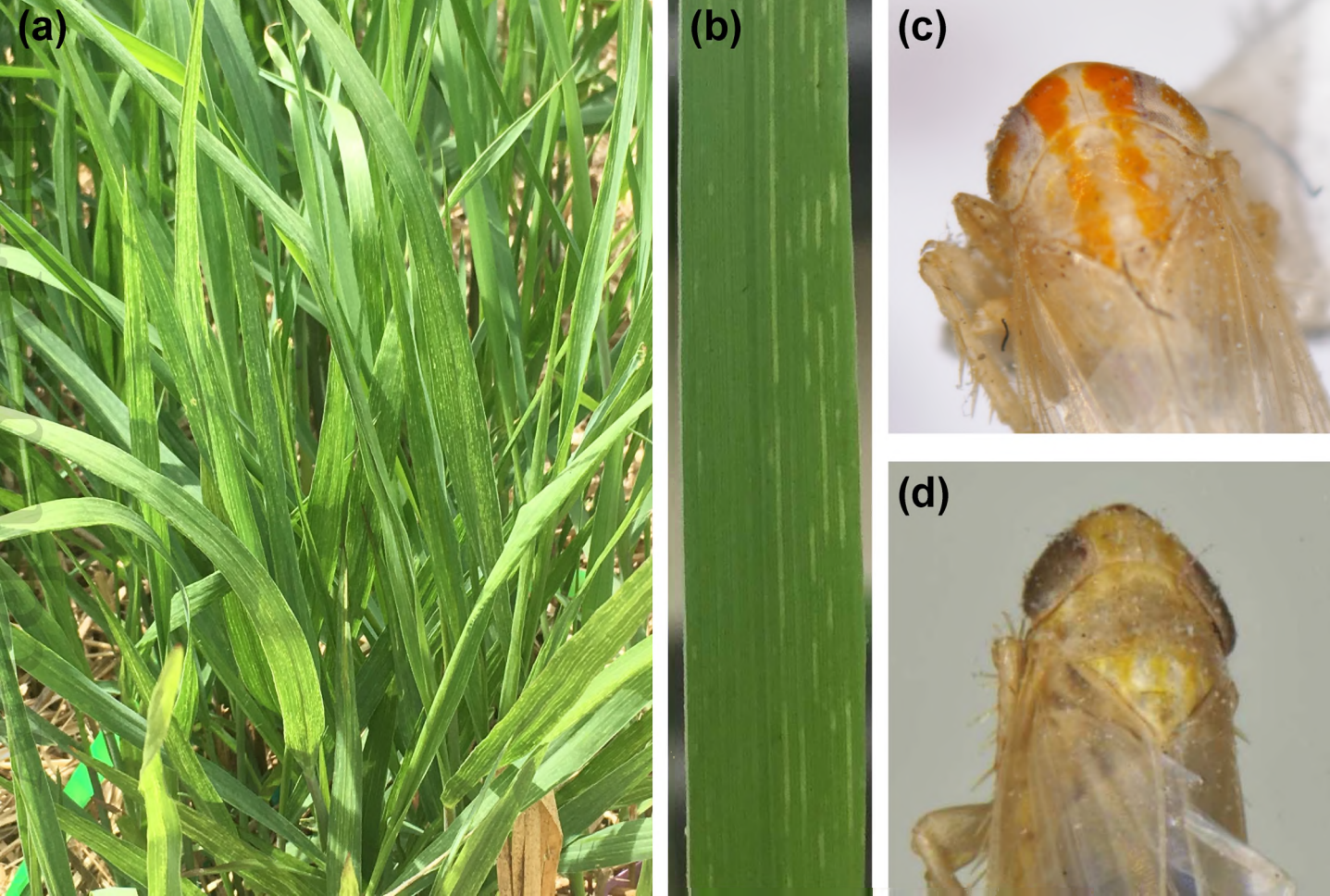
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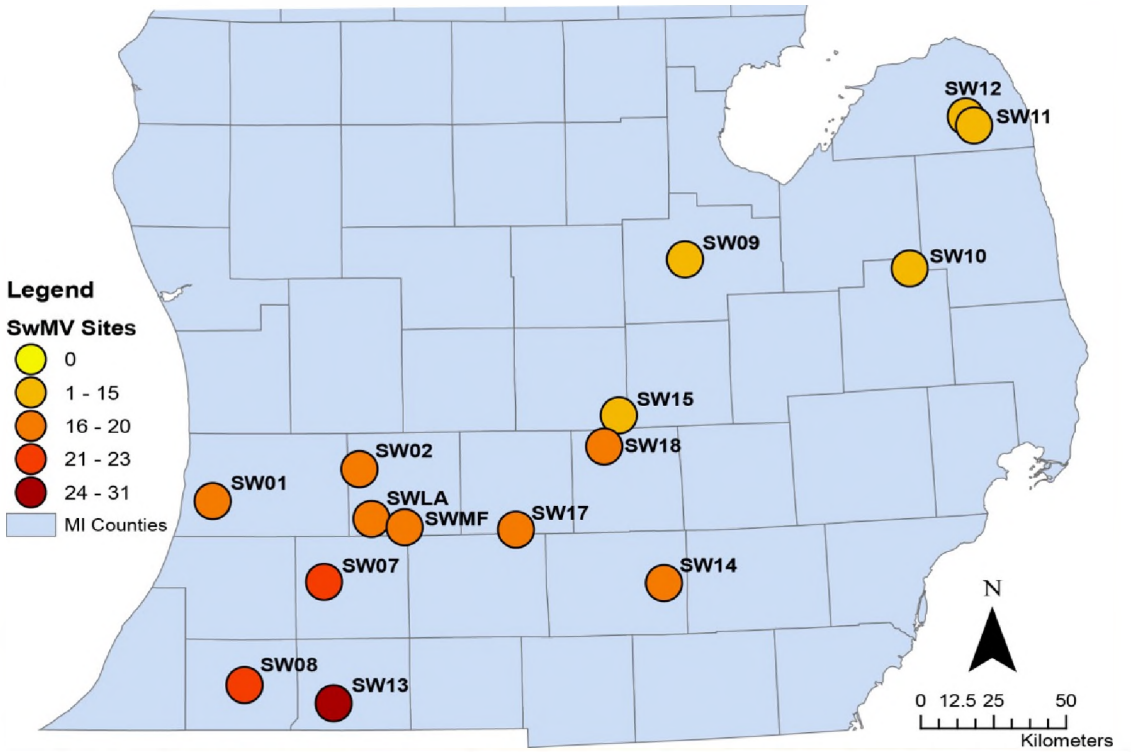

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SW02	.	.	.	.	.	.	.	1	1	1	2	3	3	3	3	2	19
SW07	.	.	.	.	1	1	1	1	1	1	2	3	3	3	3	2	22
SW08	.	.	.	.	1	1	1	1	1	1	2	3	3	3	3	3	23
SW09	.	.	.	.	.	.	.	.	.	.	1	2	2	2	2	1	10
SW10	.	.	.	.	.	.	.	.	.	.	1	2	3	3	3	3	15
SW11	.	.	.	.	.	.	.	.	.	.	1	2	3	3	3	3	15
SW12	.	.	.	.	.	.	.	.	.	.	1	2	3	3	3	3	15
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SW17	.	.	.	.	.	.	1	1	1	1	2	3	3	3	3	2	20
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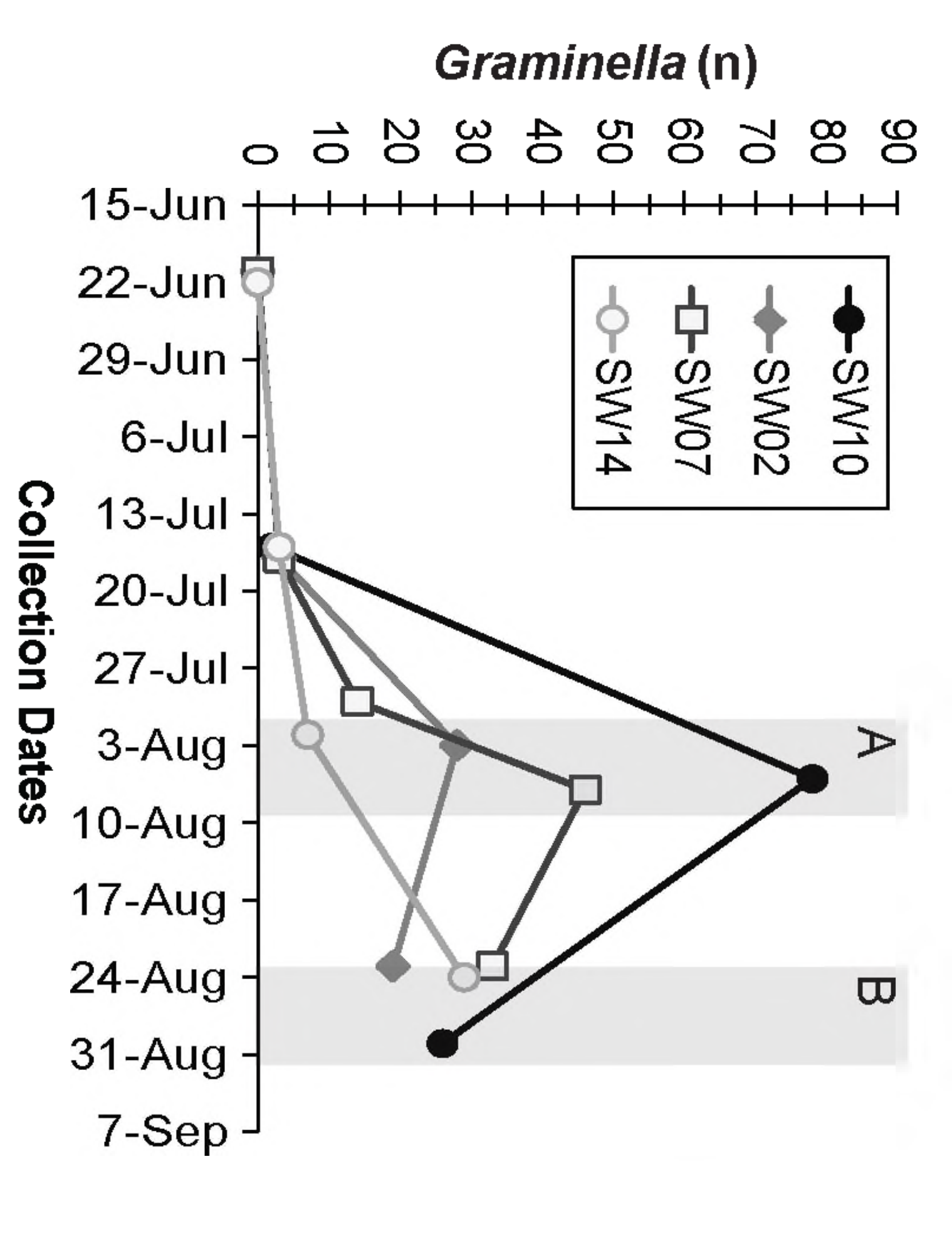
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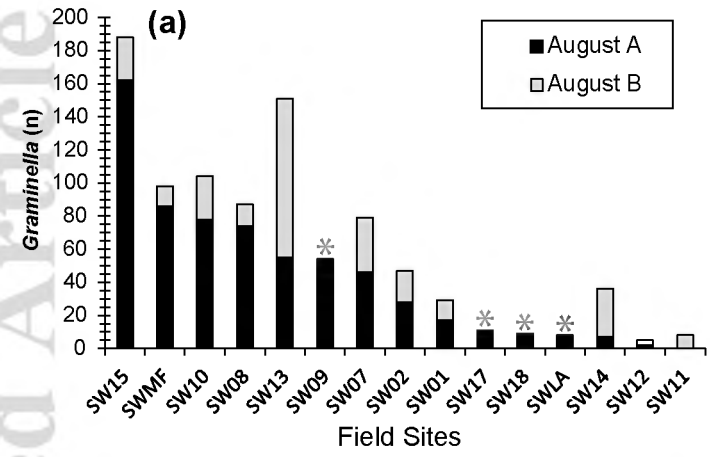

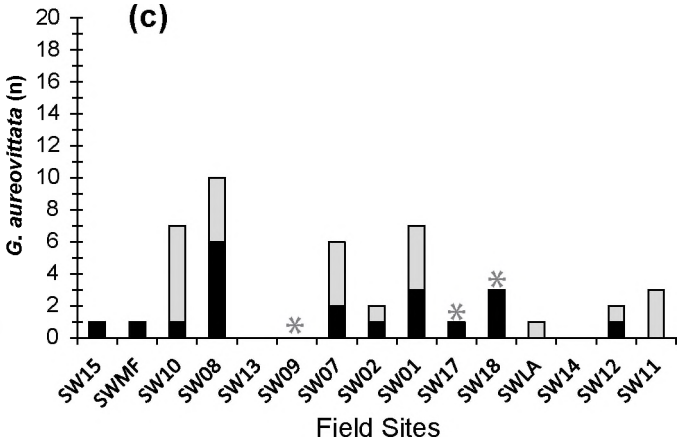

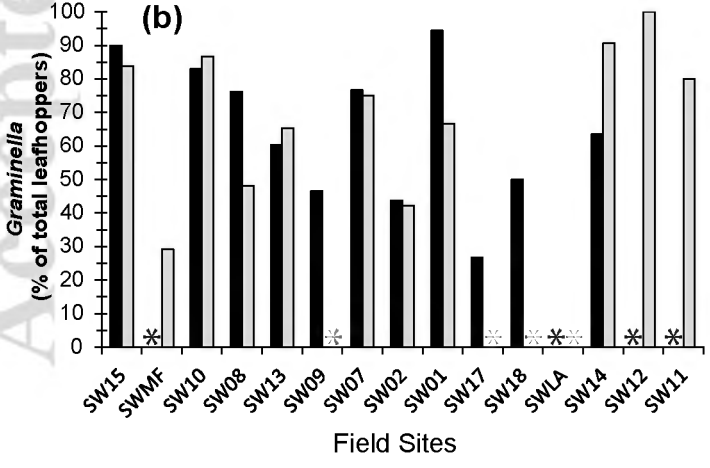

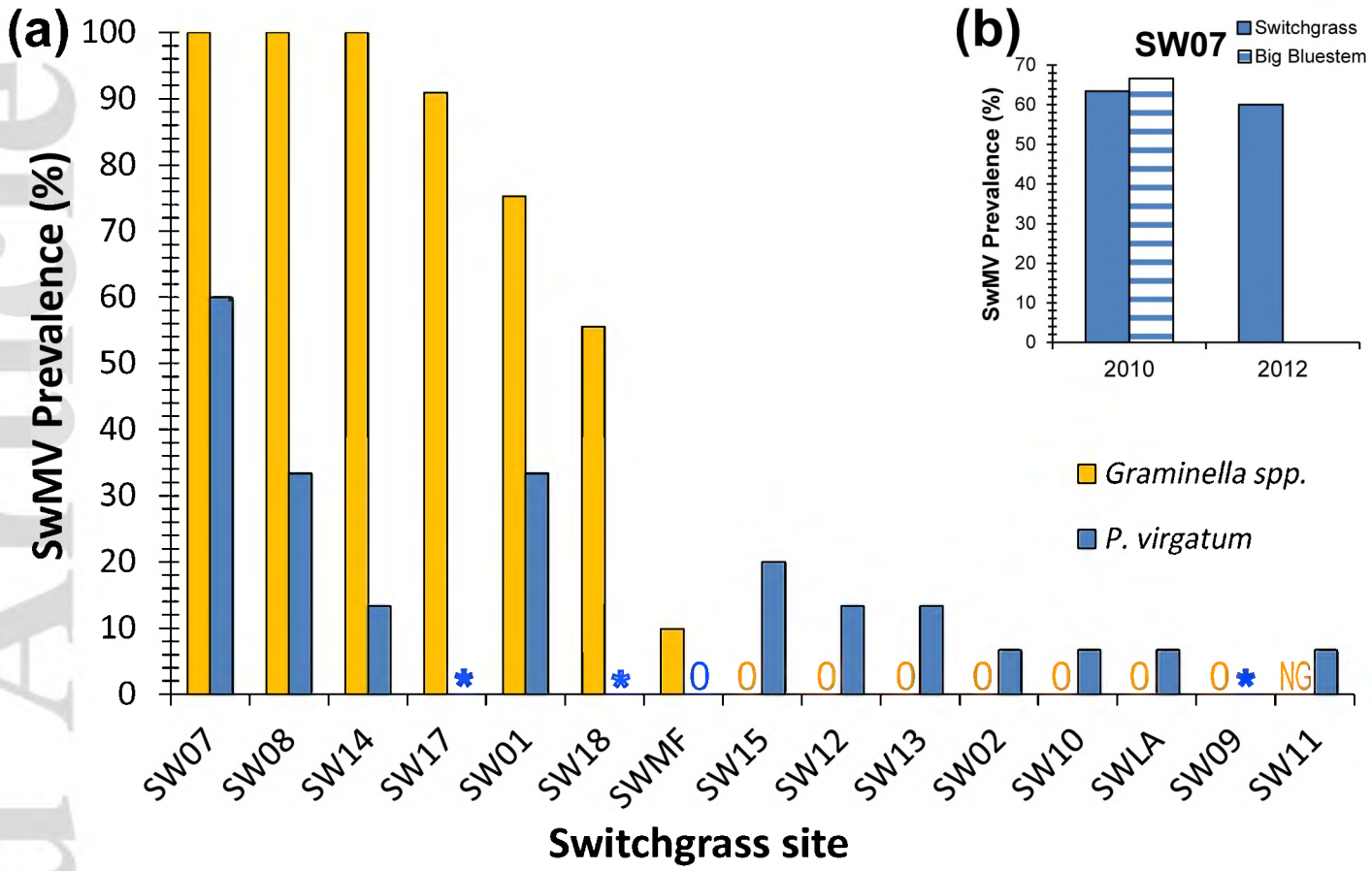

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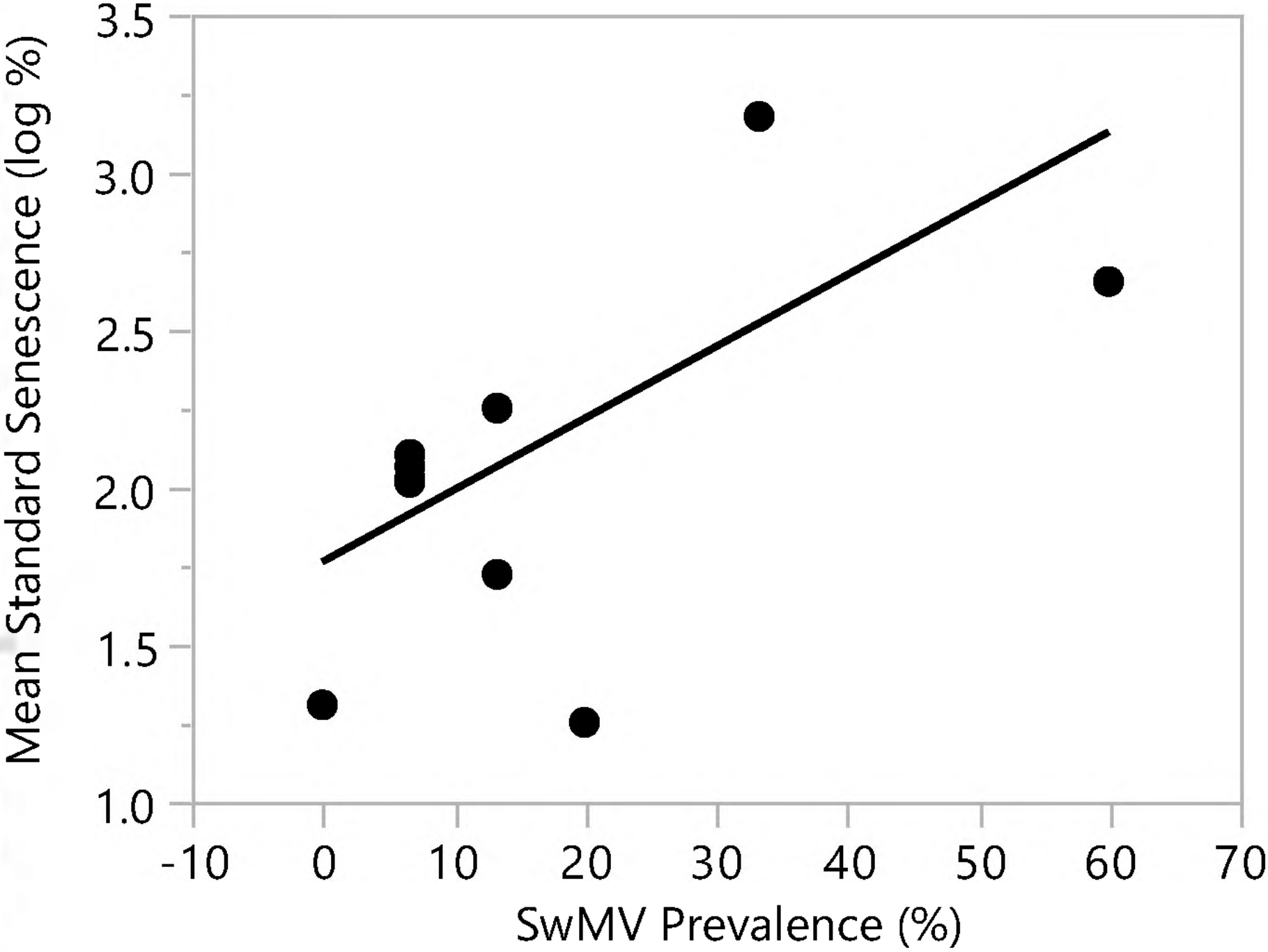
Mean Standard Senescence (log %)
3.5
3.0
2.5
2.0
1.5
1.0
-10
0
10
20
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40
50
60
70
SwMV Prevalence (%)