PRAIRIE GRASSES FOR BIOFUELS AND ECOLOGICAL RESTORATION: MODIFICATIONS TO NATIVE SPECIES AND THEIR PLACE IN HUMAN-MODIFIED LANDSCAPES

By

Abbie C. Schrotenboer

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ABSTRACT

PRAIRIE GRASSES FOR BIOFUELS AND ECOLOGICAL RESTORATION: MODIFICATIONS TO NATIVE SPECIES AND THEIR PLACE IN HUMAN-MODIFIED LANDSCAPES

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In the 19th century, North American prairie grasses were plowed under for agricultural expansion. Nearly two hundred years later, in the 21st century, these same grasses have become key species in ecological restoration and appear likely to become equally important as novel bioenergy crops that provide ecosystem services. In these new roles, these plants' traits are being changed through selective pressures, both intentionally and inadvertently. At the same time, human activities have fundamentally altered the landscapes in which these species are now found. To understand how propagation and selection pressures alter native prairie grasses, I conducted a suite of field and greenhouse experiments to test plant interactions with abiotic and biotic factors. Since the use of marginal lands is a major focus of sustainable bioenergy development, I established common gardens of both wild-collected and propagated populations of *Andropogon gerardii* (big bluestem) and *Schizachyrium scoparium* (little bluestem) in fertile loamy soils and marginal sandy soils to measure growth traits. To assess biofuel-valuable traits in *Panicum virgatum* (switchgrass), I examined relationships between growth rates and biomass recalcitrance to digestion in five populations, ranging from near-wildtype to highly selected cultivars, grown in a common garden. I then evaluated relationships between these traits and *P. virgatum* susceptibility to a widely distributed group of aphid-transmitted Poaceae viruses (Luteoviridae: *Barley and cereal yellow dwarf viruses*, B/CYDVs), which cause major yield losses in cereal crops. Significant trait differences were evident among populations of all grasses

studied. Propagated populations of *A. gerardii* exhibited a stronger plastic response to growth on fertile soils in both biomass production and reproductive investment than did wild-collected populations. In *P. virgatum*, fast growth in highly selected populations was further associated with increased susceptibility to virus infection. These findings highlight the need to evaluate potential selection trade-offs between fast growth and other plant traits, particularly as native grasses are further modified for biofuel-valuable traits.

Interactions of perennial grasses with pathogens are particularly important because these grasses can serve as reservoirs of pathogens that spill-over into other landscape compartments. To assess the importance of community and landscape context on pathogen interactions with novel biofuel crops, I examined B/CYDV and aphid vector pressure in two potential crops comprised of native grasses—*P. virgatum* and mixed prairie—and a current biofuel crop—maize.   In collaboration with a team of researchers, I evaluated a suite of community, landscape, and environmental variables for associations with vector abundance and virus incidence. Greater landscape diversity around study fields was associated with reduced aphid incidence, and consistent regional patterns of aphid and virus pressure were evident based on several measures. Landscape changes associated with biofuel production have the potential to either amplify or moderate aphid and virus pressures through effects on landscape diversity and land cover types.

Human influence on native grasses is exerted across a broad range of scales. At the population scale, particular plant traits are favored over others, and at a larger scale, human land use practices directly and indirectly affect biotic and abiotic interactions with these grasses. These influences have important consequences, especially when major restructuring of landscapes is a likely outcome of biofuel development and when generalist pathogens, such as B/CYDVs, are involved.

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INTRODUCTION

Overview

Human influence permeates the world today, from massive changes to the earth's land cover (Kareiva et al. 2007) to selection pressures placed on microorganisms (Palumbi 2001). This influence affects places and species that are normally considered to be wild. In my research, I seek to understand how prairie species have been and are being altered as they are used in two human endeavors—ecological restoration and biofuel production—and in turn how these changes may further alter ecological interactions at the landscape scale. Not only does landscape context affect the ecology of native species, but landscape context will also change dramatically as more lands are dedicated to biofuel crop production in order to meet human demand for energy (Perlack et al. 2005, Field et al. 2008).

Humans seek to shape nature in ways that provide benefits and reduce risks to themselves (Kareiva et al. 2007), but these actions often give rise to unintended consequences that threaten the sustainability of natural systems and ecosystem services. Ecological restoration seeks to return systems to a more natural state of structure and function (Palmer et al. 2006), often with the goal of providing ecosystem services (Hobbs and Harris 2001) that were lost when original communities were converted for other human uses. While the goal may be a self-sustaining, natural ecosystem, restoration usually involves intensive human management, from establishing plants to conducting regimented burns or mowing treatments (Packard and Mutel 1997). Thus, anthropogenic influences can affect the entire system—from site characteristics to species composition to genetic diversity within a species. My work investigates the nature of changes to plant traits that occur, sometimes intentionally and sometimes inadvertently, as plants are propagated for human uses.

As well as for restoration, native plant species are also being considered as potential new crops to serve as sources of cellulosic material for bioenergy production. Much attention in this area has focused on *Panicum virgatum* L. (switchgrass), a major component of the tallgrass prairie in North America, which may be grown in monoculture (Schmer et al. 2008) or in polyculture with other prairie species (Mulkey et al. 2008). Perennial biofuel crops have the potential to help meet human demand for energy while also requiring fewer inputs than traditional crops (such as maize (*Zea mays*)) and providing ecosystem services (Robertson et al. 2008). Human influence may be more obvious when species are being modified for use as bioenergy crops than when used for restoration purposes. For example, studies and selection programs are pursuing the possibility of increasing plant productivity and tissue digestibility in *P. virgatum* (Casler et al. 2002, Boe and Lee 2007, Casler 2010), including through genetic transformation (Fu et al. 2011).

The modification of native species for human purposes such as restoration and bioenergy production represents a new wave of plant domestication. As has been true historically, domestication may provide immediate benefits to humans, but problematic unforeseen consequences may also arise. Trade-offs may occur during selection such that varieties with biofuel-valuable traits, such as increased efficiency with which biomass can be converted to ethanol, may lose ability to deal with environmental stressors, both abiotic and biotic. These trade-offs may have implications for the wider landscape, through gene flow or the dispersal of pests and pathogens. For example, varieties selected for rapid growth at the expense of plant defenses could become a reservoir for pests and pathogens to spillover into other landscape compartments.

While human-designed ecosystems and agriculture may place pressures on the surrounding landscape, the landscape also influences interactions within restoration sites and biofuel fields. For example, landscape diversity can increase natural pest regulation and abundances of natural enemies (Bianchi et al. 2006). Landscape changes for bioenergy production could decrease landscape diversity if a single biofuel crop is dominant (Landis et al. 2008, Landis and Werling 2010), or such changes could increase landscape diversity if a variety of novel crops are grown.

My research investigates the effects of selection through propagation on native prairie species, with the specific goals of understanding how such selection has altered (1) plant responses to marginal soils, (2) biofuel-valuable traits, such as plant productivity and biomass recalcitrance, and (3) interactions with generalist plant viruses and their aphid vectors. Such changes to plant traits may have “bottom-up” effects for community and landscape dynamics. However, I also investigate the potential for “top-down” interactions: how does the larger community and landscape affect pest and pathogen pressure among native species?

Background

PRAIRIE VEGETATION IN THE MIDWESTERN UNITED STATES

Prior to European settlement, much of the Midwestern United States was a mosaic of forest, savanna, and tallgrass prairie that comprised an area sometimes referred to as the prairie peninsula. Transeau (1935) outlined the prairie peninsula as occurring through most of Iowa and Illinois, much of Minnesota, Wisconsin, and Missouri, and into parts of Indiana, Ohio, and

Michigan. The region of the prairie peninsula is defined by climate, but other environmental factors, such as fire frequency and soil type, contribute to the specific locations of prairie within the mosaic of vegetation types (Davis 1977). Although General Land Office (GLO) surveys provide records of the historical extent of prairies and savannas, much of our knowledge about their pre-settlement composition must be based on small remnant areas because GLO surveys rarely recorded specific herbaceous species (Rodgers and Anderson 1979). Warm-season grasses, including *Andropogon gerardii* Vitman (big bluestem), *Schizachyrium scoparium* (Michx.) Nash (little bluestem), *Panicum virgatum* L. (switchgrass), and *Sorghatrum nutans* (L.) Nash (Indian grass), are usually the dominant species in a community that also includes a diverse mixture of forbs and a few cool-season grasses. However, there is some evidence that in the most eastern regions of the prairie peninsula, such as Ohio, forbs had been more prevalent than the warm-season grasses (Kalisz and Boettcher 1990, Mack and Boerner 2004).

Since the time of European settlement (around the 18th century), the prairie peninsula has been drastically altered through direct conversions of the land and through changes to natural disturbance regimes. Prairies were plowed for the cultivation of crops; this occurred most rapidly on dry prairie but was slower to occur in the wetter areas of rich, fertile soils that required drainage (Whitney 1994). In addition, settlers sometimes introduced heavy grazing by livestock, and eventually many areas that were formerly flooded in the spring were drained to make way for human use of the landscape (Transeau 1981). Fire suppression policies also followed in the wake of settlement (Packard and Mutel 1997). Without fire, cool-season grasses, many of which were introduced from Europe, were able to dominate over native warm-season grasses (Whitney 1994), and woody species were also able to invade formerly herbaceous sites (Vale 1982).

Few remnants of the prairie peninsula’s grassland communities remain. In Michigan, for example, the Department of Natural Resources (DNR) estimates that approximately 99% of the state’s prairies and savannas either have been converted to agriculture or have undergone succession to forest (Michigan DNR 2006), and other regions suffered similar losses. For instance, McLean County, Illinois, has no prairie remnants today, despite the fact that the 1820’s GLO land survey found it to be nearly 90% prairie (Rodgers and Anderson 1979). An assessment of remnant prairies in Illinois revealed that the majority are found on land unsuitable for agriculture, and few remnants are found on the fertile black soils of the state (White 1981); particular land uses, such as cemeteries and railroad right of ways, have provided havens for prairie communities in addition to those lands unsuitable for agriculture (Whitney 1994).

ANTHROPOGENIC INFLUENCE ON PRAIRIE SPECIES AND COMMUNITIES

Prairie restoration

Today, prairie vegetation is being planted in many areas, on public and private lands, to meet conservation goals and to provide ecosystem services. Prairie restoration provides habitat for grassland-nesting birds and other rare and endangered species, such as the Karner Blue butterfly (Pickens and Root 2008). Several prairie and savanna community types are considered critically imperiled at the state or global level, so restoration can aid in the conservation of communities as well as species (O'Conner 2006). Grasslands also provide services such as soil conservation and carbon sequestration (Jastrow 1987, Baer et al. 2002).

Although many state lands in the Midwest are being restored, prairie restoration in this region occurs largely on private lands. These lands include those owned by nature centers and conservancy organizations as well as by individual landowners who have become involved through federally- and state-funded programs. For instance, the USDA's Conservation Reserve Program (CRP) has provided for the planting of 2.6 million hectares of native grassland throughout the United States (Barbarika 2006), with a large portion planted in tallgrass prairie vegetation. To prevent erosion and provide wildlife habitat, this federal program provides cost-sharing, technical assistance, and annual payments to private landowners for establishing permanent vegetation cover on their agricultural land (Gray and Teels 2006). State governments have also provided assistance in establishing prairie vegetation through Landowner Incentive Programs (LIPs).

Restoration projects on lands owned by the state or by private conservation organizations usually select sites for restoration that are believed to have been prairie prior to European settlement, and these projects often use wild-collected seed sources from the local area when possible. However, many of the programs for private lands select areas without regard to prior vegetation, focusing instead on marginal agricultural lands in areas at risk with regard to soil erosion or wildlife habitat. Plant material is often purchased from large seed companies that produce cultivars of the warm-season prairie grasses. Concerns have been raised about the potential negative consequences of using cultivated and non-local plant materials for the restoration of native communities (Knapp and Dyer 1998, Lesica and Allendorf 1999).

Native species developed for bioenergy

In recent years, ethanol produced from cellulosic biomass sources has been considered as a possible source of liquid fuel that would reduce greenhouse gas emissions and attain a greater net energy yield than grain ethanol (Lemus and Lal 2005). Monocultures of the prairie grass *P. virgatum*, as well as diverse prairie communities, are being considered as possible sources of biomass. Species-rich prairie communities, in particular, have potential to provide not only biofuel feedstocks but also ecosystem services such as wildlife habitat, carbon sequestration, and soil conservation, without requiring high energy inputs (Robertson et al. 2008). In some cases, diverse prairie may be more productive than switchgrass monocultures, as seen on the low fertility abandoned fields studied by Tilman et al. (2006). However, in other environments, *P. virgatum* monocultures may be more productive, and the payoff from slightly higher inputs (such as fertilizer) may improve economic returns (Schmer et al. 2008).

INFLUENCE OF CULTIVATION OR COMMERCIAL PROPAGATION

Concerns for restoration

Humans can alter the genetic composition of native species through cultivation, which can affect plant performance and persistence in a restoration. During cultivation, artificial selection for particular desirable plant traits may occur, as well as unintentional selection due to site conditions and propagation techniques. Often the seed sources used in restoration of the major prairie grasses are the same seed sources used for forage production. Thus, many of the grasses planted in restorations are cultivars developed for traits valued for forage, such as productivity, leafiness, and vigor (Alderson and Sharp 1995).

Although short term seed increases are often considered to have negligible genetic impact, work suggests that even one generation of propagation may alter the resulting genetic composition (McKay et al. 2005). The effects of propagation are various and likely to accumulate as time spent in cultivation increases. For example, seed propagation is likely to select for plants that produce prolifically near harvest time and for plants with a height range that corresponds to method of harvest (Knapp and Dyer 1998). Selection can also be caused by the environmental conditions of the propagation site. At the regional scale, cultivars have often been propagated under different climatic conditions (e.g., different precipitation regimes and growing season length) than those of potential restoration sites. At a more local scale, the choice of soil type for a propagation field will alter selective pressures. If a fertile soil is used, then plants that are able to best utilize abundant resources may be selected for. However, this selection may favor plants that are less adapted to the conditions of many restorations, which may be in areas deemed unsuitable or only marginally suitable for agriculture. In addition, fertilizers often are used in order to maintain profitable seed yields in warm-season grasses (Cornelius 1950), which will further emphasize responsiveness to high nutrient levels.

Selection for biofuel-valuable traits

As switchgrass and other prairie grasses are bred for biofuel feedstocks, plant traits that allow for high biomass production and for increased efficiency of conversion to ethanol will be favored. Switchgrass breeding in the past has often selected for greater *in vitro* dry matter digestibility (IVDMD) for use as forage (Vogel et al. 1991, Vogel et al. 1996), and higher IVDMD is also associated with increased efficiency in converting cellulosic feedstocks to ethanol (Lorenz et al. 2009, Bals et al. 2010). One of the main factors affecting IVDMD is lignin content; lignin causes recalcitrance to enzymatic digestion (Gomez et al. 2008), and its digestion can result in inhibitors that interfere with fermentation. Lignin content can be altered through structural changes to plant growth forms, since stems generally have higher proportions than leaves, and through changes to cell wall composition (Sarath et al. 2008). Mutant lines with reduced or altered lignin content have been identified in several crop species, such as maize, sorghum, and pearl millet; some occur naturally while others have been created through mutagenesis. These mutant lines often have higher digestibility, and there is some evidence of greater palatability for mammalian grazers (Li et al. 2008).

EFFECTS OF SELECTION ON ALLOCATION TRADE-OFFS

As certain traits are selected for or against during propagation or breeding, this either requires re-allocation of carbon to certain structures or compounds (such as the production of more seeds) or releases carbon that can be redirected elsewhere (such as when lignin concentrations are reduced). These trade-offs between different plant functions and structures could have unintended consequences for plant fitness and performance during plant breeding for bioenergy crops.

A key trade-off plants face is between growth and defense. In general, fast growth is associated with short lifecycles and reduced investment in defense, whereas slow growth is associated with longevity and greater investment in defense mechanisms (Coley et al. 1985). Plants with high relative growth rates typically produce fewer secondary metabolites (Herms and Mattson 1992). The growth-defense trade-off has a simple genetic basis in some cases; *Arabidopsis thaliana* with an allele for constitutively activated defenses had slower growth rates than plants without that allele (Todesco et al. 2010). High growth rates have also been connected with reduced disease resistance in agriculture; for example, fruit trees under high fertilization rates (resulting in higher growth rates) produced fewer phenolic compounds and had greater disease incidence (Leser and Treutter 2005). Thus, selecting prairie grasses for increased productivity may come at the cost of defenses against herbivores and pathogens.

Trade-offs in allocation among plant structures also is of concern. During domestication and continued breeding of cereal grasses, some gains have been made in the overall biomass production of the species (particularly in maize), but most gain in seed production is due to changes in resource allocation (Buckler et al. 2001). Increased allocation to seed production involves not only a shift in the use of photosynthate but also changes in the nitrogen budget. Because seeds require proportionally more nitrogen than vegetative growth, nitrogen uptake must be increased or nitrogen in vegetative tissues must be decreased (Sinclair 1998). Such changes in allocation in the prairie grasses could reduce plant fitness in the context of a restoration environment, where soil nitrogen levels may be low and stresses high, due to reduced investment in vegetative growth or belowground storage.

Modifications to plant cell walls to increase the efficiency of producing ethanol will also likely affect non-target traits. Changes to lignin content could have ramifications for plant growth, survival, and disease resistance. For example, in the maize *brown midrib-3* mutant, which has lower lignin, yields of both grain and stover (stalks and leaves) were reduced (Lee and Brewbaker 1984), and yield reductions have also been noted in sorghum and pearl millet lines with reduced lignin (Pedersen et al. 2005). Switchgrass lines that had undergone either one or three cycles for increased IVDMD had higher mortality rates and lower productivity than did the parental line and a line bred for lower IVDMD. Low survival rates were attributed to winterkill (Casler et al. 2002). When planted in denser stands, however, it appears that surviving switchgrass individuals can compensate for winterkilled plants, probably through increased tillering (Vogel et al. 2002). There is also recognition that breeding for lower lignin content could reduce resistance to pathogens and insect damage (Li et al. 2008, Sarath et al. 2008), but this is an area that needs further study.

Despite problems associated with altered lignin production, careful monitoring and selection has the potential to overcome some fitness hurdles associated with low lignin. Some species actually have increased productivity; a transgenic poplar line with a mutation that specifically affects xylem lignin had seedlings that grew larger and produced more cellulose than the control line (Hu et al. 1999). Whether this kind of response is restricted to woody species remains to be seen, but selection for yield and survival can counteract some of the negative impacts seen in low-lignin populations of herbaceous plants. In alfalfa, a low-lignin population had much greater mortality than a high-lignin line (Casler et al. 2002); however, when the progeny from the surviving low-lignin plants were grown, their survival rate was similar to high-lignin plants (Casler et al. 2002). When switchgrass half-sib families were developed from a population with high IVDMD, some families had very high mortality while others did not differ from the low IVDMD population (Vogel et al. 2002).

In some cases, greater value of the product may compensate for reductions in biomass. In transgenic alfalfa lines, two low-lignin lines had greater efficiency for ethanol conversion than a control line even when the low-lignin lines were not acid pre-treated and the control was (Chen and Dixon 2007). Thus, if the conversion efficiency is high enough, smaller plants may still yield more fermentable sugars at lower cost than would larger plants with normal lignin levels.

INFLUENCE OF GEOGRAPHIC ORIGIN ON SEED SOURCES

In addition to the effects of cultivation, the geographic source of plants used in a restoration plays a role in plant genotypes and phenotypes. Different taxa may vary in their level of genetic and phenotypic differentiation among populations, and a few studies have investigated these differences in prairie species. In a study comparing remnant and restored prairie populations of *A. gerardii* using Random Amplified Polymorphic DNA (RAPD) analysis, no significant difference was found in genetic diversity or composition between remnant and restored populations in Ohio (Selbo and Snow 2005). The highly outcrossing, wind-pollinated breeding strategy of the species may lead to limited genetic differentiation among populations. However, another study of *A. gerardii* did find that Illinois populations grouped separately from Arkansas populations in principle component analysis of RAPD loci (Gustafson et al. 1999). In the case of a prairie forb, *Dalea purpurea*, Illinois and Kansas populations grouped separately from each other based on allozyme data, and within Illinois remnant populations, geographic distance was negatively associated with genetic similarity (Gustafson et al. 2002).

The use of genetic markers in these studies cannot reveal differentiation for genes of adaptive significance. Thus, it is also important to consider phenotypic differences, which provide more insights into adaptive significance. Phenotypic differences also demonstrate how plants respond to an environment of interest. McMillan (1964, 1965) has shown that three of the major prairie grasses, *A. gerardii*, *S. nutans*, and *S. scoparium*, vary in several phenotypic traits when clones from multiple provenances were grown in a common garden in Texas. Plants exhibited considerable differences in size, flowering, timing of spring activity, leaf and culm growth rates, and timing of dormancy. These studies indicate that phenotypic traits and genetic markers can differ among populations for certain prairie species. However, it is not clear how important such differences are to plant fitness in a prairie restoration or a biofuel crop.

Although differences among populations are often assumed to be adaptive and the result of selection, differences could also be due to genetic drift, and in this case, local plants may not be the best adapted to their environment (Knapp and Rice 1996). Thus, relative fitness of the plants should be evaluated. In common garden experiments with grassland species, local plants have been documented to produce greater biomass than non-local plants (Gustafson et al. 2004a, Vergeer et al. 2004), have increased survival (Jones and Hayes 1999), and flower more frequently (Vergeer et al. 2004). However, the reverse has also been found to be true in some cases, such that, at least in the initial year or two after establishment, non-local plants may produce greater biomass (Smith et al. 2005), have increased survival, and have greater seed production (Jones and Hayes 1999). Millar and Libby (1989) note that in the case of economically valuable tree species, sometimes non-local genotypes will fail to establish, but in other cases, non-local genotypes will do well until an infrequent disturbance or disease outbreak. Thus, local adaptation may not be immediately apparent during the limited years of a study if it depends on relatively rare events.

PRAIRIE SPECIES AND INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Climatic effects: Temperature and moisture regimes

Precipitation plays a major role in determining the distribution and behavior of prairie species. A dry air mass typically extends over the prairie peninsula. In many years the moister Gulf air mass can bring rain during the summer, but when this does not occur, drought results (Geis and Boggess 1968). The eastern extent of the tallgrass prairie receives less growing season precipitation than the more central states to the west, and these areas have less winter snow cover than areas of predominantly deciduous forest to the north and south (Transeau 1935). In areas with a relatively long growing season, flowering in the prairie community as a whole was highly correlated with precipitation (Kebart and Anderson 1987).

In more northern areas of the tallgrass prairie where growing season is shorter, temperature plays a greater role in plant phenology (Kebart and Anderson 1987). Flowering in these northern communities was more correlated with temperature. In common gardens of the major prairie grasses in various climates, plants from more northern origin flower earlier than those from southern origin (McMillan 1959, 1965, Olson 1986). Since plant populations from different areas within the tallgrass region are attuned to different environmental cues, long distance movement of plant material could lead to inappropriate timing of plant responses.

At the landscape level, differences in moisture availability can affect plant growth and productivity. In an assessment of upland and lowland areas in the Konza Prairie in Kansas, plants from upland areas had a greater degree of water stress (as measured by leaf xylem pressure potential) (Knapp et al. 1993). Lowland plants had greater leaf area, which created a greater water demand, and these plants experienced more water stress than would have been expected based on soil moisture information alone .

Prairie productivity is controlled to a large degree by an interaction between precipitation and fire regime. In years with high precipitation, recently burned areas will have greater aboveground net primary productivity (ANPP) than unburned areas, but this trend is reversed in dry years. In a dry year the thatch in unburned areas can aid in conserving soil moisture (Knapp et al. 1998), but thatch may also limit precipitation throughfall and nutrient additions to the soil (Seastedt 1985). In biofuel fields, which are annually harvested, the removal of plant biomass may mimic some of the effects of fire.

Nutrient Availability

Nutrient availability adds another layer of complexity to environmental controls on ANPP in the prairie. In the Konza prairie discussed above, areas that are burned only periodically can produce a greater pulse of ANPP after a burn than annually burned areas because nitrogen has accumulated in the soil (Knapp et al. 1998). Nitrogen addition typically increases *A. gerardii* biomass in lowland areas (Turner and Knapp 1996) and decreases midday water potential of *A. gerardii* and *S. nutans* (Silletti and Knapp 2001).

At the community level, increased nutrient availability can reduce diversity. In prairie plots with added nitrogen, a cultivar of *P. virgatum* increased in dominance and decreased forb diversity (Baer et al. 2005). This indicates that increasing nutrient levels may work against some restoration goals by limiting the establishment of desired prairie species. In old fields, nitrogen additions reduced forb diversity and establishment of *A. gerardii* seedlings, due to increased biomass of other species (Foster and Gross 1998).

Genotype x environment interactions

Interactions between genotype and environment can affect the productivity of potential biofuel sources. In *P. virgatum*, interactions between cultivar type and growing conditions make it difficult to predict which cultivar will be most productive (Casler and Boe 2003). Evaluation of various cultivars and remnant populations of switchgrass has shown only limited evidence for local adaptation, and authors have suggested that appropriate cultivars be chosen based on the ecoregion and hardiness zone of intended planting sites (Casler and Boe 2003, Casler 2005) rather than strict local seed zones.

Interactions with the environment can also affect concentrations of cellulose and hemicellulose in plant tissue, which provide substrate for producing ethanol. For example, warm-season grasses grown at several Canadian sites showed a positive relationship between accumulated thermal time and the proportion of hemicellulose produced (Jefferson et al. 2004). Thus, biofuel feedstock quality can be affected by the location of crop production fields.

IMPORTANCE OF THE BIOTIC ENVIRONMENT

Pathogen activity in restored vegetation and biofuel crops

Plantings of prairie grasses, whether in a restoration or a biofuel crop field, may be more vulnerable to pathogens than existing stands of native vegetation because a young population age-structure is created. Large-scale restoration often begins with the removal of any existing vegetation, followed by the application of seed or young plants to the site. Since seed and seedling stages have the highest disease-related mortality in natural ecosystems (Gilbert 2002), susceptible hosts are present in high numbers after the initial planting of a site.

The source of plant material may also affect disease prevalence depending on whether populations carry disease resistance to local pathogens. If material is from local sources, the plants may be adapted to the pathogens they encounter and suffer fewer ill effects, whereas non-local plants may be naive and more vulnerable (Millar and Libby 1989). Alternatively, non-local plants often represent a novel host that the pathogen is unable to infect successfully. For example, in a highly inbreeding woodland species, *Amphicarpaea bracteata*, infection by a host-specific fungus was greatest among plants re-introduced into their site of origin as opposed to plants from populations as close as one kilometer distant (Parker 1985). Dispersal of both host and pathogen should be considered when determining whether local adaptation is likely to play a role in plant-pathogen interactions in a given restoration since dispersal and thus population structure may differ between the two (Thrall et al. 2001).

Barley and cereal yellow dwarf viruses

One group of pathogens that has the potential to interact with grasses in prairie restorations and biofuel fields is the *Barley and cereal yellow dwarf viruses* (*Luteoviridae*:

BYDVs and CYDVs, hereafter B/CYDVs). B/CYDVs are aphid-vectored viruses known to infect over 100 species in the Poaceae family (Irwin and Thresh 1990). This group of viruses is economically important since it infects several major cereal crops. Incidence of B/CYDVs was negatively correlated with yields in both oats and wheat in Australia (McKirdy and Jones 2002), and infection reduced yields in wheat by up to 39% in an Illinois field study (Perry et al. 2000). B/CYDVs cause symptoms such as stunting, discoloration, reduced flower production, and in some cases sterility. The symptoms and their severity depend upon the viral strain, the host plant species, and the host plant's condition (D'Arcy 1995). Infection usually leads to phloem degeneration, which alters the movement of carbohydrates in the plant; carbohydrates accumulate in leaf tissue and are more scarce in root tissue (Jensen and D'Arcy 1995).

Strains of B/CYDVs are known to infect wild prairie grasses. In a early study by Stoner (1976), *S. scoparium* was shown to be susceptible to a virus transferred using *Rhopalosiphum padi* L. (bird cherry-oat aphid), but *A. gerardii* and *P. virgatum* were not. The strain of BYDV was most likely either a PAV or an RPV species, which are the strains normally transmitted by *R. padi* (Irwin and Thresh 1990). In a study of grasses in the Flint Hills of Kansas, plants were tested for the five recognized species of B/CYDV. In *A. gerardii* and *P. virgatum*, BYDV-MAV was the predominant infecting species, and in *S. scoparium*, BYDV-SGV was the most common (Garrett et al. 2004). Virus was not detected in any *S. nutans* individuals. None of the prairie species carried BYDV-PAV, which was commonly found in wheat crops in the area. However, little is known about the fitness effects of infection on prairie grasses.

Most of the information about B/CYDVs in natural ecosystems comes from California grasslands. In both observational and experimental studies, seven species of native California grasses showed reduced growth and fecundity when infected with B/CYDVs, and survivorship was reduced in some species (Malmstrom et al. 2005a, 2006). These viruses have also been implicated in the decline of native perennial grasses and the invasion of European annual grasses in the California grasslands through apparent competition. Exotic annuals increase both aphid density and the incidence of viral infections in native grasses (Malmstrom et al. 2005b). Recent modeling work has indicated that in the absence of virus, the perennial grasses would be the dominant species, but when B/CYDVs are present in the system, the exotic annuals become dominant (Borer et al. 2007). However, not all viruses have negative effects on plant hosts, and in fact, some have mutualistic interactions under stressful environmental conditions (Gibbs 1980, Márquez et al. 2007, Xu et al. 2008). Thus, negative fitness effects as a result of infection cannot be assumed in all cases.

Community and landscape influences on pathogen and vector pressure

Pathogens and vector prevalence can be affected by species diversity and composition within a site as well as by the vegetation in the surrounding landscape. At the community level, some pathogens have lower incidence in polyculture or high diversity plots than in monoculture or low diversity plots (Piper et al. 1996, Knops et al. 1999). However, the combination of particular species, or types of species, can also increase pathogen pressure. For example, in California grasslands, susceptible annual hosts can increase the incidence of B/CYDV infection in perennial grasses (Malmstrom et al. 2005b, Borer et al. 2007).

At the broader scale, different land cover types around a site may act as sources or sinks of pathogens, vectors, or predators of vectors. Greater levels of maize agriculture in the landscape has been linked to increased pressure from the crop pest *Aphis glycines* (soybean aphid) due to lower levels of natural enemies (Landis et al. 2008). Other perennial land cover types, such as forest and grassland, on the other hand, can increase the prevalence of lady beetles that prey upon aphids (Gardiner et al. 2009b).

POTENTIAL LANDSCAPE INTERACTIONS

Gene flow

The use of cultivated or non-local plant populations in the landscape can result in altered intraspecific and interspecific interactions. This may occur within a restoration site, in which various species are competing, but it can also occur as a result of dispersal and gene flow. Introduced genotypes in a restoration or biofuel field may not remain restricted to the planting site, moving through either seed or pollen dispersal. Particularly in the case of the prairie grasses, which are highly out-crossing, wind-pollinated species, pollen dispersal is likely if con-specifics are within dispersal ranges. The long dispersal distances of grasses have been documented in *Agrostis stolonifera* (creeping bentgrass), which has been shown to cross-pollinate with plants up to 21 km distant (Watrud et al. 2004).

When local genotypes come into contact with introduced genotypes, genetic swamping can occur within a species, such that the introduced genotypes come to dominate the gene pool. The risk for genetic swamping is particularly high when remnant populations are small and restored populations are large (Knapp and Dyer 1998). Hybridization between local and introduced genotypes can also have consequences. In some cases heterosis, which is an increase in fitness when two different populations or species form a hybrid, is seen in the F_1 generation of a cross (Fenster and Galloway 2000, Keller et al. 2000). However, outbreeding depression is another possible outcome, in which fitness of the offspring is reduced due to the loss of local adaptation or the disruption of co-adapted gene complexes (Keller et al. 2000, Montalvo and Ellstrand 2001, Hufford and Mazer 2003).

Community dynamics can also be altered through the introduction of non-local or cultivated genotypes. Restorations often fail to meet goals for forb establishment, and highly competitive grass genotypes could contribute to this failure. In a chronosequence of prairies restored using cultivar grasses, forbs decreased over time, and grasses increased (Baer et al. 2002). In an experimental prairie restoration in Kansas, a cultivar of *P. virgatum* dominated the experimental plots, and the greater the percent cover of this species, the lower the plot diversity. The authors recognized that the lack of appropriate disturbance contributed to the dominance of the grass species, but they postulated that use of cultivars was also a factor (Baer et al. 2005). Remnant populations could also be affected if gene flow is occurring from planted fields to remnant grasslands. For example, if cultivated grass genotypes are found to show more vigor and higher growth rates than local genotypes, gene flow from these non-local genotypes could lead to more aggressive growth by grasses in remnant populations (Lesica and Allendorf 1999). This may increase the grass population, but may have negative effects on other species in the remnant.

Pathogen and vector interactions in the landscape

If anthropogenic changes to native species alter interactions with pathogens and vectors, these changes could have implications for pathogen dynamics at the landscape and regional scale. Wild grasses formerly dominated the Midwestern and central United States, and today these areas are largely devoted to cultivated grasses—cereal crops, such as wheat (*Triticum aestivum*) and maize. Thus, widespread biofuel plantings of cultivar grasses that are susceptible to pathogens and vectors could result in pathogen spillover into the surrounding landscape, into both food crops and what little is left of remnant grasslands. Although disease and pests have been recognized as possible problems in biofuel crops (Royle and Ostry 1995, Gustafson et al. 2003, de Vries et al. 2010), the potential for interactions with other vegetation types is only beginning to be recognized (Spencer and Raghu 2009).

CHAPTER 2

ANTHROPOGENIC INFLUENCE ON TRAITS OF NATIVE GRASSES USED IN PRAIRIE RESTORATION AND BIOFUELS

Abstract

Native plant species experience anthropogenic pressures whenever humans design ecosystems or manage native plant communities. Here, we consider whether human selection, either deliberate or unintentional, has altered traits of native prairie grasses used in restorations and considered for novel bioenergy crops. Propagated plants may have an altered ability to deal with environmental stresses, which is of key importance for restorations and biofuel crop production on marginal lands. To examine how propagation affects native species, we compared performance of multiple wild-collected and propagated populations of two North American prairie grasses, *Andropogon gerardii* and *Schizachyrium scoparium*, in common gardens on fertile and marginal soils. In addition, we evaluated plant root fractions, responses to nutrient additions in marginal soils, and effects of intraspecific competition in fertile soil. Evidence of anthropogenic selection was most striking in the growth of propagated *A. gerardii* populations, which grew notably larger and invested more in reproductive structures than did wild-collected populations on agricultural quality soils. However, propagated populations performed similarly to wild-collected populations on marginal soils. Fertilization of sandy soils had no evident effect on *S. scoparium* and actually reduced plant size in *A. gerardii*. Differences in flowering phenology were evident between local and non-local populations. When exposed to environmental stresses as small seedlings, some non-local populations had reduced biomass and survival in comparison with local populations. Our findings demonstrate that selection pressures due to cultivation methods deserve careful consideration and highlight the need to evaluate

potential trade-offs between fast growth and other plant traits, particularly as native grasses are further modified for biofuel-valuable traits.

Introduction

In North America, native plants are increasingly used in horticulture and forage production, where introduced species have traditionally dominated (Vogel and Moore 1993, Anderson 2000). In addition, native species are being cultivated for new purposes such as ecological restoration (Packard and Mutel 1997, Knapp and Dyer 1998) and biofuels (Jefferson et al. 2004, Stork et al. 2009). Broadened use of native plants raises questions about human influence on species that are generally still considered “wild” (Rolston 2004). How do traits differ among wild-type and propagated populations? What implications do these differences have for the use of propagated populations in restoration and bioenergy crops?

In ecological restoration, much attention has focused on differences in plant material derived from local or from non-local source populations. In this context, *local* may indicate distance from a project site, such as 10 miles or 100 miles (Kurtz 2001), or a broader area, such as an ecoregion (Jones 2005). All other factors being equal, local source populations are generally preferred in restoration (Mortlock 2000, Gustafson et al. 2005), both to preserve extant diversity and because these populations may exhibit local adaptation. Local plants may have greater survival or reproductive output than non-local ones (McGraw 1987, Joshi et al. 2001), although experimental evidence has been mixed (Jones and Hayes 1999, Smith et al. 2005). However, it can be difficult to obtain sufficient quantities of local propagules for large projects when nearby remnant populations are small or uncommon, and acquiring wild-collected see can be time consuming and costly (Smith et al. 2007). To increase local seed supplies, commercial producers in some areas collect seed from local wild populations and propagate them at nearby facilities to amplify supplies.

Alternatively, when local plant materials are limited, project managers may chose to use seeds from non-local sources. Non-local sources may also be preferred if (i) local populations have been subject to inbreeding depression, due to isolation or small size (Vergeer et al. 2004); (2) a mix of genotypes might provide resiliency in the face of climate change (Harris et al. 2006, Broadhurst et al. 2008); or (3) specific traits are desired in plant material, such as for biofuel production. However, the choice of non-local plant materials can prove costly if survivorship or performance in the new environment is poor. Non-local material may also pose risks of unwanted gene flow into remnant populations (Gustafson et al. 2005, McKay et al. 2005, Broadhurst et al. 2008) and of outbreeding depression (Keller et al. 2000, Montalvo and Ellstrand 2001). Moreover, if a large quantity of seed is sought, sources may be limited to large commercial producers that offer named *cultivars* selected for vigor or other specific characteristics. Non-local, wild-collected seed is much less available and thus rarely used.

As this situation makes evident, choice of local or non-local material is complicated by financial considerations and by limitations in availability of wild-collected seed, which can lead to the consequent use of plant material that has been propagated and thereby subjected to human selection. This selection may occur deliberately, as in the development of named cultivars, or inadvertently, through the processes of seed collection and subsequent propagation. Here, we examine several key questions that require further investigation: In what ways and to what extent do the traits of wild-collected and propagated native plants differ? How do any observed trait differences influence the performance of populations in environments with different stresses? If a population is commercially propagated in a relatively resource-rich agricultural environment, how will this influence the population's performance in more natural, resource-limited environments? For example, how do propagated populations respond to marginal soils and competition?

Studies of propagation effects in wild plants are few and largely focus on the potential for changes to genetic diversity and allele frequencies at neutral markers (Larson et al. 2000, Gustafson et al. 2004b, Dolan et al. 2008). For example, in *Elymus trachycaulus* subsp. *subsecundus* (awned slender wheatgrass), seed from a multi-site composite showed significant loss of allelic diversity between G_0 and G_2 populations (Ferdinandez et al. 2005). However, studies comparing genetic differentiation for traits important to plant fitness between propagated and wild-collected populations are scarcer. A few studies of *Panicum virgatum* L. (switchgrass) have included both wild-collected and cultivar populations but focused mainly on differences in population performance across climatic gradients (Hopkins et al. 1995, Casler et al. 2007) rather than specifically addressing the effects of propagation. A one-year study by Gustafson et al. (2004a) suggested that cultivars of *Andropogon gerardii* Vitman (big bluestem) might produce as much or more biomass than plants grown from local wild-collected seed, but did not examine longer-term performance or the effects of environmental stresses.

In this case study, we investigate differences in genetic-based traits of wild-collected and propagated populations of two North American prairie grasses, as evident in common gardens in different soils. We focus on the warm-season prairie grasses *Andropogon gerardii* and *Schizachyrium scoparium* (Michx.) Nash (little bluestem), which are dominant tallgrass prairie species (Weaver 1968) used in most Midwestern prairie restorations. Interest in native grasses for prairie restoration and for biofuel feedstock production is rising (Sanderson et al. 2004, Stork et al. 2009) because prairie communities can provide multiple ecosystem services, such as soil conservation (Jastrow 1987, Udawatta et al. 2008), carbon sequestration (Baer et al. 2002, Kucharik 2007), and bird habitat (Giuliano and Daves 2002). Recognition of these benefits and of the threatened status of prairie communities (O'Conner 2006) has prompted restoration efforts on both public and private lands. Bioenergy crops based on either monocultures of prairie grass or mixed prairie assemblages could provide many of these services that first-generation biofuels, such as maize and soybean, do not, and they may perform well on marginal lands (Lemus and Lal 2005, Tilman et al. 2006).

Plant performance in stressful environments is a key factor to consider because many projects involving native species will occur on marginal lands. Biofuel cropping targets lands that are less suited to agriculture in order to limit competition with food production (Robertson et al. 2008), and initiatives that provide funds for grassland restoration, such as the Conservation Reserve Program in the United States, focus on highly erodible lands, which tend to be less productive than less erodible lands (Lubowski et al. 2006). Despite this focus on growing plants on marginal lands, propagation of most plants generally occurs under higher-resource conditions.

Under more fertile conditions in propagation fields, any selection that occurs will be acting on phenotypes produced under high resource availability. Plants adapted to fertile soils may grow rapidly and out-compete others during plant establishment. Larger plants with greater seed production can also contribute more heavily to mechanized seed harvests (Knapp and Dyer 1998).

In the Midwest, cultivars often are the primary seed sources for large-scale prairie grass restorations, and the same is likely to be true of biofuel plantings. Since many prairie grass cultivars originate from the western tallgrass prairie region, including Kansas, Nebraska, and Iowa (Alderson and Sharp 1995), the populations introduced to the central Midwest are both propagated and non-local. Because the duration and intensity of cultivation can vary considerably depending upon seed source, we here use *cultivar* to describe a named variety of a species that has been subject to long-term (>20 years) artificial selection. *Propagated* populations are those that have been grown for seed production for any time. *Wild-collected* populations, in contrast, are those in which seed was harvested from wildland populations with no commercial management. We use *population* broadly to describe (i) seed collected from wild plants at a single site and (ii) commercial seed packaged as one type, which may have either single-site or multi-site origin. In our study, all local populations are from within Michigan and the ecoregion identified as the Southern Michigan/Northern Indiana Drift Plains in the US EPA's level III ecoregion map (USEPA 2007).

Our broad goals are to provide insight into anthropogenic influence on wild plants and to help inform choices made about appropriate seed sources for restoration and biofuels. In this study, we addressed the following specific questions with an integrated suite of field experiments:

(1) To what extent do propagated populations of *A. gerardii* and *S. scoparium* differ from wild-collected populations in growth rates, reproductive investment, and mortality? Are trait differences evident even in populations propagated without intentional selection? Do differences persist over time? We expected propagated populations to produce greater biomass and invest more in sexual reproduction than wild-collected populations.

(2) Do wild-collected and propagated populations of *A. gerardii* and *S. scoparium* perform equally well on both high-quality and marginal soils? Has propagation under high fertility conditions altered plant responses to poorer soils? There are two competing hypotheses concerning the effects of selective pressures during propagation: (H_1) intrinsic trade-offs in plant

performance lead to selection for plants that are most fit in more fertile environments, or (H_2) the conditions of propagation exert selective pressure on plant plasticity. In the case of H_1, we would expect propagated plants to perform better than wild-type ones on fertile soils and wild-type plants to perform better on marginal soils. In the case of $H_{2,}$ we would expect both propagated and wild-type plants to perform similarly on marginal soils but propagated populations to show larger performance gains on fertile soils than wild-type plants.

(3) How do additional stresses associated with field plantings, including environmental stresses and those from plant competition, influence the survival of wild-collected and propagated *A. gerardii* populations? If a mix of source populations is used, differential survival could limit establishment of particular genotypes. We expected that *A. gerardii* populations that had the highest growth rates in fertile soils of a common garden setting would have the greatest survival when exposed to intraspecific competition. For example, rapidly growing plants from propagated populations could capture large proportions of light and other resources, thereby limiting resources available to plants from wild-collected populations.

(4) Is there evidence of local adaptation to soil characteristics? We expected plants from “home site” soils most similar to experimental plot soils to exhibit higher productivity and greater reproductive investment than plants from more dissimilar soils.

Methods

COMMON GARDEN STUDY ON DIFFERENT SOIL TYPES

To compare the performance of wild-collected and propagated prairie grass populations, we established a common garden experiment with six populations of *A. gerardii* and five populations of *S. scoparium* in Michigan in June 2006. We included Michigan wild-collected, Michigan propagated, and non-local propagated populations (Table 2.1). In this first experiment, we based the choice of plant materials on those likely to be used in restoration practice. Because non-local, wild-collected seeds are generally not considered for restorations, we did not include them in the initial common garden study although they were introduced into later experiments. Local, propagated seed was purchased from Native Connections (Three Rivers, MI, USA), where production fields were irrigated during droughts and received minor nitrogen fertilization (~17 kgN/ha) during the first year of establishment only (J. Stewart, pers. comm). Non-local cultivars were purchased from Star Seed (Beloit, KS, USA), where seed production typically involves annual nitrogen applications.

To test whether populations differed in response to soil type, we established plants in both sandy and loamy soils at each of two locations: (1) Michigan State University's Horticulture Teaching and Research Center in East Lansing, MI, (42.7ºN 84.5ºW; East Lansing plots) and (2) a commercial farm in Mason, MI (42.6ºN 84.4ºW; Mason plots). The sites are located about 11 km apart in the central lower peninsula of Michigan in Ingham County. Loamy soil plots contained greater organic matter and more nutrients than sandy soil plots, with the exception of phosphorus, which was elevated in both soils at the Mason location (Table 2.2). At both locations, plot-specific gravimetric soil moisture was 3–5 times greater in loamy soils than in sandy soils based on assessments in August and September, 2007 (data not shown, $P < 0.05$).

We started cold-treated seeds in the greenhouse in 3B Mix potting media (Conrad Fafard Inc., Agawam, MA, USA) in April 2006 and planted the seedlings as 12.7 cm plugs in June 2006 in a randomized complete block design using 2 x 7 m blocks (Mason plots 19 blocks per soil type; East Lansing loam, 38 blocks; and East Lansing sand, 18 blocks) (n = 94 per population x

Table 2.1. Prairie grass populations examined in this study. Michigan populations are named by county of origin with the exception of specific varieties. CG = common garden study (2 sites, 2 soil types), R:S = root fraction study (in sandy soil at Mason site), and SS = small seeding study (in loamy soil at Mason site).

Population	Type	Experiments	Geographic origin and propagation information	Seed acquisition
A. gerardii				
Kalamazoo[1]	Wild, local	R:S, CG, SS	Fort Custer State Recreation Area, MI (42.33ºN 85.32ºW)	Field collected fall 2005
Oakland	Wild, local	R:S, CG, SS	Golden Preserve, Oakland land conservancy, MI (42.75ºN 83.51ºW)	Field collected fall 2005
Washtenaw	Wild, local	R:S, CG, SS	Dexter-Huron Metro Park, MI (42.34ºN 83.88ºW)	Field collected fall 2005
Konza	Wild, non-local	R:S, SS	Konza prairie, Geary County, KS	Field collected fall 2007
Ionia	Propagated, local	R:S, SS	Michigan, propagation fields in Portland, MI	Purchased from Michigan Wildflower Farm, fall 2007
St. Joseph[2]	Propagated, local	R:S, CG, SS	Southwestern Michigan, propagation fields near Constantine, MI	Purchased from Native Connections, spring 2006
'Pawnee'[3]	Propagated, non-local	R:S, CG, SS	Southeastern Nebraska, named variety, propagated in Kansas	Purchased from Star Seed, spring 2006
Prairieview[4]	Propagated, non-local	R:S	Indiana, propagated in Indiana	Provided by Rose Lake Plant Materials Center
'Rountree'[5]	Propagated, non-local	R:S, CG, SS	Western Iowa, named variety, propagated in Kansas	Purchased from Star Seed, spring 2006

Table 2.1 (cont'd)

Population	Type	Experiments	Geographic origin and propagation information	Seed acquisition
S. scoparium				
Oakland	Wild, local	R:S, CG	Golden Preserve, Oakland land conservancy, MI (42.75ºN 83.51ºW)	Field collected fall 2005
Berrien	Wild, local	R:S, CG	Grand Mere State Park, MI (42ºN 86.53ºW)	Field collected fall 2005
Kalamazoo[1]	Wild, local	CG	Fort Custer State Recreation Area, MI (42.33ºN 85.32ºW)	Field collected fall 2005
Konza	Wild, non-local	R:S	Konza prairie, Geary County, KS	Field collected fall 2007
St. Joseph[2]	Propagated, local	R:S, CG	Southwestern Michigan, propagation fields near Constantine, MI	Purchased from Native Connections, spring 2006
'Aldous'[3]	Propagated, non-local	R:S, CG	Eastern Kansas, named variety, propagated in Kansas	Purchased from Star Seed, spring 2006
'Blaze'[6]	Propagated, non-local	R:S	Kansas and Nebraska, named variety	Purchased from Sharp Bros. Seed, spring 2008
Prairieview[4]	Propagated, non-local	R:S	Indiana, propagated in Indiana	Provided by Rose Lake Plant Materials Center

[1] Collected from a restored prairie with some remnant grasses but also planted with seeds collected from within the Fort Custer grounds.

[2] Started with Southlow germplasm which originated from collections in 1999 and are local to the region of study.

[3] Began development in the 1930's

[4] Germplasm of both species has been released as Prairieview

[5] Began development in the 1980's

[6] Began development in the 1950's

Table 2.2. Soil characteristics of the common garden study plots, seed collection sites, and the Native Connections propagation fields. Common garden soils were tested prior to planting and fertilization, and all other sites were tested in spring of 2008. Nutrient analysis conducted by the Soil and Plant Nutrient Laboratory (Michigan State University, East Lansing, MI).

Site type	Location	Organic matter (%)	Total N (%)	Nitrate (ppm)	Ammonium (ppm)	P (ppm)	Cation exchange capacity (meq /100 g)	pH	Soil type	Soil taxonomic class
Loamy garden plots	East Lansing	2.3	0.12	10.40	1.75	55.0	10.6	6.9	Loam, sandy loam	Capac loam
	Mason	4.9	0.26	2.40	0.60	495.0	14.3	7.5	Sandy loam	Capac loam
Sandy garden plots	East Lansing	0.8	0.03	2.50	1.00	39.0	6.0	7.9	Loamy sand	(Excavated fill)
	Mason	1.1	0.04	1.40	0.80	157.0	2.8	6.9	Sand	Spinks loamy sand
Collection locations	Oakland	2.5	0.12	0.49	2.48	21.3	5.2	6.2	Loamy fine sand	Riddles sandy loam
	Washtenaw	2.2	0.09	1.14	3.14	14.7	6.2	7.9	Loamy sand, sandy loam	Boyer loamy sand
	Kalamazoo	2.6	0.15	2.20	2.05	27.3	5.8	7.7	Loamy sand	Oshtemo sandy loam
	Berrien	1.5	0.06	1.25	1.45	5.5	3.0	7.9	Loamy sand	Granby loamy fine sand
Propagation field	St. Joseph	1.4	0.07	0.44	1.43	60.3	4.5	6.6	Loamy sand	Oshtemo sandy loam, Spinks loamy sand

11 populations, experiment-wide). We irrigated the plots during summer 2006 to ensure establishment; plots were regularly weeded from 2006 to 2008. In 2009, we applied mowing treatments, not presented here, to two-thirds of the blocks (see Phillippo & Malmstrom, in prep.).

To determine whether nutrients limited plant growth on sandy soils, we fertilized portions of sandy soil plots in summer 2008 (Yr 3). We applied Lesco Professional (John Deere, Troy, MI, USA), a slow release fertilizer which included urea nitrogen (24% N, 0% P, 11% K, 2.9% S, and 3% Fe), to randomly selected blocks in the sandy soil plots (containing both *A. gerardii* and *S. scoparium*) at both East Lansing and Mason and left others as unfertilized controls. We chose a fertilizer without phosphorus because soil analysis indicated that phosphorus levels were already within recommended levels for agronomic soils. We fertilized on 3 June and 27 July 2008, each time at a rate of 49 kgN/ha and 22 kgK/ha, as recommended by the fertilizer manufacturer (total rate: 98 kgN/ha, 44 kgK/ha).

Traits measured

Throughout, we refer to the year of planting and initial establishment (2006) as Yr 1; 2007, Yr 2; and so on. To assess growth and reproductive investment in the field, we measured height, basal area, tiller number, time of first flowering, and inflorescence number during Yrs 1 and 2. In Yr 3, we measured plant height, basal area, and inflorescence number to assess effects of fertilization. In October of Yrs 1, 2, and 4, we also harvested *A. gerardii* plants by clipping at a height of 6 cm above the ground, and dried harvested biomass for 3 d at 70˚C. We harvested *S. scoparium* biomass only in Yrs 2 and 4; in Yr 1, plants were too short (many tillers ~ 6 cm) to harvest. In Yr 1, we weighed total aboveground biomass (both vegetative and reproductive) to assess overall growth and seedhead biomass alone to assess reproductive investment. To assess whether propagated populations may have increased investment in individual seeds (which might account for observed differences in germination rates (A. Schrotenboer, personal observation)), in Yr 2, we determined average weight of an individual seed by weighing 25 seeds of *A. gerardii* or 50 seeds of the relatively small-seeded *S. scoparium*.

Plant survival data from 2009 were gathered in spring prior mowing to treatments; data from 2009 on plant growth are from un-mowed controls only (6 blocks from all plots, except the East Lansing loamy soil, which had 14 blocks).

ROOT FRACTION IN SANDY SOILS

To evaluate whether anthropogenic selection had altered root fraction in these grasses, we planted an additional common garden experiment in 2008 for destructive root sampling. We started plants in the greenhouse and planted them as plugs into the Mason sandy soil plot; we chose a sandy soil for this experiment because we expected differences in belowground investment to be particularly important on marginal soil. Plants were arranged in a randomized complete block design with 1 m spacing between plants on 10 June 2008 (n = 9 per population x 16 populations). All populations included in the original common garden were included, with the exception of the Kalamazoo population of *S. scoparium,* for which the seed supply was limiting. We also included additional seed sources of both species to provide a greater spectrum of wild-collected and propagated seed sources (Table 2.1), including a wild-collected, non-local population from Kansas (Konza). On 10 October 2008, we harvested all plants by digging in a circle (r ~ 20 cm, determined by harvesting roots of test plants) around each individual until the roots had been freed. We hand-washed roots and oven dried whole plants for 3 d at 70˚C. We separated and weighed above- and belowground biomass.

INFLUENCE OF STRESS ON SEEDLINGS OF *A. GERARDII*

To assess population and survival and performance under a broader suite of environmental stresses, we established an additional field experiment that evaluated survival and growth of densely planted small seedlings of A. gerardii. Plants were younger (~ 3 week old), and were transplanted from much smaller plugs than in the common garden experiments (2.5 cm cell depth, versus 12.7 cm). To deliberately establish competition, we planted *A. gerardii* individuals much closer together (0.12 m spacing, versus 1 m in the common garden), planted prairie forbs, and allowed weeds to grow after an initial establishment period. For this experiment, we planted 2.25 m^2 plots in the Mason loamy soil plot with seedlings of *A. gerardii* and two Michigan genotype prairie forbs, *Coreopsis lanceolata* and *Lupinus perennis* (Michigan Wildflower Farm, Portland, MI, USA). We chose the loamy soil for this experiment because competition was expected to be greater in this environment based on higher growth rates of *A. gerardii* in loamy soils than sandy soils in the common garden study. Buffer strips of 0.5 m width separated each plot. Each competition plot contained four populations of *A. gerardii*: all propagated populations (n = 3 plots), all wild-collected populations (n = 3 plots), or two of each type (n = 4 plots). In plots with both population types, populations were randomly assigned to two of the four plots. We planted 31 individuals from each of the four populations of *A. gerardii* in a randomized design in each plot along with 10 individuals each of *C. lanceolata* and of *L. perennis*. Seedlings were planted in 12 rows and 12 columns with 12 cm spacing between each plant.

Prior to planting, seedlings were grown in square plug trays (1.4 x 2.5 cm) (Landmark Plastic Corp., Akron, OH) and filled with Baccto Professional Potting Mix (Michigan Peat Co., Houston, TX). The eight *A. gerardii* populations grown included the six used in the common garden experiment as well one non-local, wild-collected population (Konza) and one additional local, propagated population (Ionia) (Table 2.1). Seeds were started in May 2007, and seedlings were planted seedlings during the first week of June 2007. Initial mortality was high (greater than 50% in some plots) due to windy, hot weather, so we replaced dead plants with new transplants the following week. We hand weeded and watered plots for the first month of the experiment; water additions were minimal, and the soil became quite dry in between waterings. In October 2008, plants were harvested, separated by population within each plot, dried, and weighed. None of the *L. perennis* individuals survived, so no further analysis is presented on this species. Population evenness was assessed using Shannon's equitability index (Shannon diversity index / maximum possible Shannon diversity index with given number of populations).

ASSESSMENT OF ENVIRONMENTAL VARIABLES AND SITE SIMILARITY

First, to assess how soil characteristics of common garden plots compared to each other and to sites of seed collection in Michigan, we conducted principal component analysis (PCA) on all quantitative soil measures in Table 2.2 using R 2.11.1 (R Foundation for Statistical Computing, Vienna, Austria). Second, to assess plant response to common garden soil characteristics, we also conducted PCA with only the garden plots—including all of the above variables as well as gravimetric soil moisture. We then used these principal component values in an ANCOVA analysis of plant performance as measured by population averages of aboveground biomass and number of inflorescences from Yr 2. Population was used as a categorical predictor, and principal component values were incorporated either with or without a quadratic term, and we used an *F* test to determine whether the linear or quadratic model was appropriate.

We evaluated how the environment of population origin influenced performance in our common garden sites. We assessed whether latitude, which serves as a surrogate for photoperiod differences, influenced flowering phenology. We used the Gower index (Gower 1971) to assess the similarity of the common gardens to population origins (e.g., seed collection locations) based on soil characteristics. The Gower index uses multiple input variables to calculate similarity coefficients between pairs of sampling units and has the advantage of using a range-standardization to account for environmental variables measured on different scales (Snelder et al. 2007). Similarity of the soil environment was based on seven quantitative variables for Michigan populations shown in Table 2.2.

STATISTICAL ANALYSIS

To determine the effect of population source (type = wild-collected or propagated), soil type, and the potential interaction of these factors on plant traits, we performed analysis of variance (ANOVA) using SAS v9.2 (SAS Institute Inc., Cary, NC). We ran analyses of common garden data as a split-plot design with soil as the split plot factor and with a random blocking factor (response = type + population(type) + soil + soil*type + soil*population(type) + location + soil*location). The Kenward-Rogers method was used to calculate degrees of freedom (Littell et al. 2006). To meet assumptions of normality, several variables were square root transformed, including number of inflorescences, number of tillers, biomass in Yrs 2 and 4, *S. scoparium* height in Yr 4, *A. gerardii* basal area in Yr 4, and root:shoot biomass measures. *Andropogon gerardii* height in Yr 4 was transformed with a power transformation using p = 2. Pairwise comparisons of growth and reproductive measures were conducted using Tukey-Kramer adjusted p-values. We conducted chi-square analyses of survivorship and phenological data using Statistix

9.0 (Analytical Software, Tallahassee, FL, USA). We also used ANOVA to assess differences in the root fraction and competition experiments. Significance was assessed using $\alpha = 0.05$, with Tukey LSD corrections used for pairwise comparisons.

We conducted correlation analysis using SAS v9.2 (SAS Institute Inc., Cary, NC) to determine whether the Gower indices were predictive of Yr 2 aboveground biomass and inflorescence production in the common gardens.

Results

ANDROPOGON GERARDII

Vegetative traits

All populations grew better on fertile soils, but propagated populations of *A. gerardii* exhibited a stronger response to fertile soils than did wild-collected populations, indicating that propagation has selected for plasticity. On sandy soils, biomass production was similar for both population types ($P = 0.54$). However, propagated populations, including cultivars, attained ~80% greater aboveground biomass on loamy soil than sandy soil ($P = 0.01$) in Yr 1; wild-collected populations attained ~40% greater biomass ($P = 0.02$) (Fig. 2.1A). This difference between propagated and wild-collected populations in their response to soil type was evident in Yr 1 and continued to Yr 4 (Fig 1A; Table A2.1). In Yr 1, propagated populations produced more tillers on loamy soil than on sandy soil ($P < 0.0001$) whereas wild-collected populations did not ($P = 0.19$) (Fig 1B). Basal area was less indicative of differences as it did not differ between population types in either Yr 2 or 4 (Table A2.1).

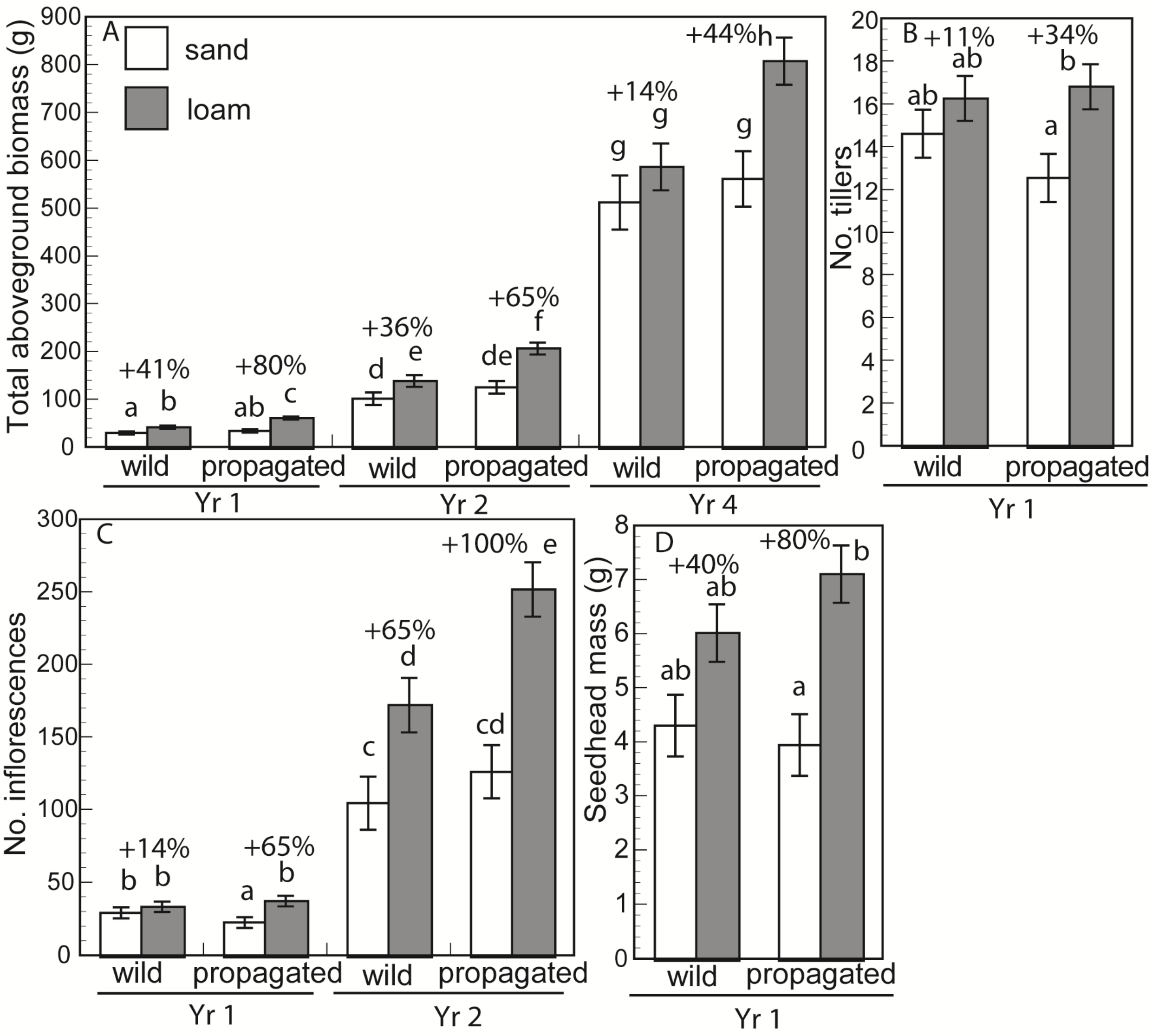

Fig. 2.1. Effect of soil type and population type on aboveground biomass of *A. gerardii* in years one (A), two (B), and three (C) and tiller number in Yr 1 (D), seedhead mass in Yr 1 (E), and number of inflorescences in Yr 2 (D). Error bars represent standard errors, and different letters indicate significant differences with each year of each graph. Numbers indicate percentage change from sandy soil to loamy soil.

Plant growth in the first years of the study strongly predicted subsequent growth. Both Yr 1 biomass ($R^2 = 0.23$, $P < 0.0001$) and Yr 2 biomass ($R^2 = .43$, $P < 0.0001$) were significant predictors of Yr 4 biomass in linear regression. Propagated and wild-collected populations exhibited similar ratios of Yr 4 to Yr 1 biomass ($F_{1,4} = 0.92$, $P = 0.39$).

Reproductive traits

Reproductive investment also demonstrated evidence of plasticity; propagated populations exhibited a stronger response to fertile soils than did that of wild-collected populations. Among propagated populations, seedheed mass was ~80% greater on loamy soils than sandy soils ($P < 0.0001$), but it was only ~40% greater among wild-collected populations ($P = 0.006$) (Fig 1C). However, seedhead mass of propagated and wild-collected populations did not differ from each other on sandy soil ($P = 0.83$). Differences between population types were also evident in the number of inflorescences produced; in Yr 2, propagated populations produced more inflorescences than wild-collected populations on loamy soils ($P < 0.0001$) but not on sandy soils ($P = 0.44$) (Fig. 2.1F).

Unlike other reproductive metrics, mass of individual seeds did not show differences according to soil or population type (Table A2.1), but inter-population differences nonetheless were evident. Cultivars invested more in sexual reproduction than other populations as indicated by seed size; seeds of the two non-local cultivars of *A. gerardii*, ‘Pawnee’ and ‘Rountree’ were the largest ($P < 0.05$). Thus, higher germination rates of the cultivars (A.Schrotenboer, personal observation) may be related to higher seed mass.

Phenology

In Yr 2, tillers emerged in late April and early May. By May 2, more *A. gerardii* on sandy soils had emerged than those on loamy soils ($\chi^2 = 26.04$, $df = 1$, $P < 0.001$). However, propagated and wild-collected populations did not differ in timing of emergence ($\chi^2 = 0.01$, $df = 1$, $P = 0.91$). Population origin, either from within Michigan or outside of Michigan, also did not affect emergence phenology ($\chi^2 = 0.38$, $df = 1$, $P = 0.85$).

Andropogon gerardii from local populations were more likely to have inflorescences early in the season (June and July) than non-local plants ($\chi^2 = 128.99$, $df = 1$, $P < 0.001$) based on Yr 2 data (Fig. 2.2A). In Yr 1, later flowering appeared to result in several immature seedheads in non-local populations, but earlier flower development among all populations in the second year allowed for most seedheads to reach maturity (A.S., personal observation).

Fertilization response

Propagated and wild-collected populations of *A. gerardii* showed similar responses to fertilization ($P > 0.05$). Contrary to expectations, fertilization reduced growth and reproductive investment of plants on sandy soils. Fertilized plants had ~15% smaller basal area ($F_{1,33} = 4.24$, $P = 0.047$) and produced ~25% fewer inflorescences ($F_{1,27} = 7.29$, $P = 0.01$) than unfertilized plants on sandy soil (Fig 3). Plant height was not significantly affected by fertilization ($F_{1,33} = 3.04$, $P = 0.09$), but the pattern suggested that fertilized plants were also slightly shorter. Fertilization effects did not persist into Yr 4 ($P > 0.05$).

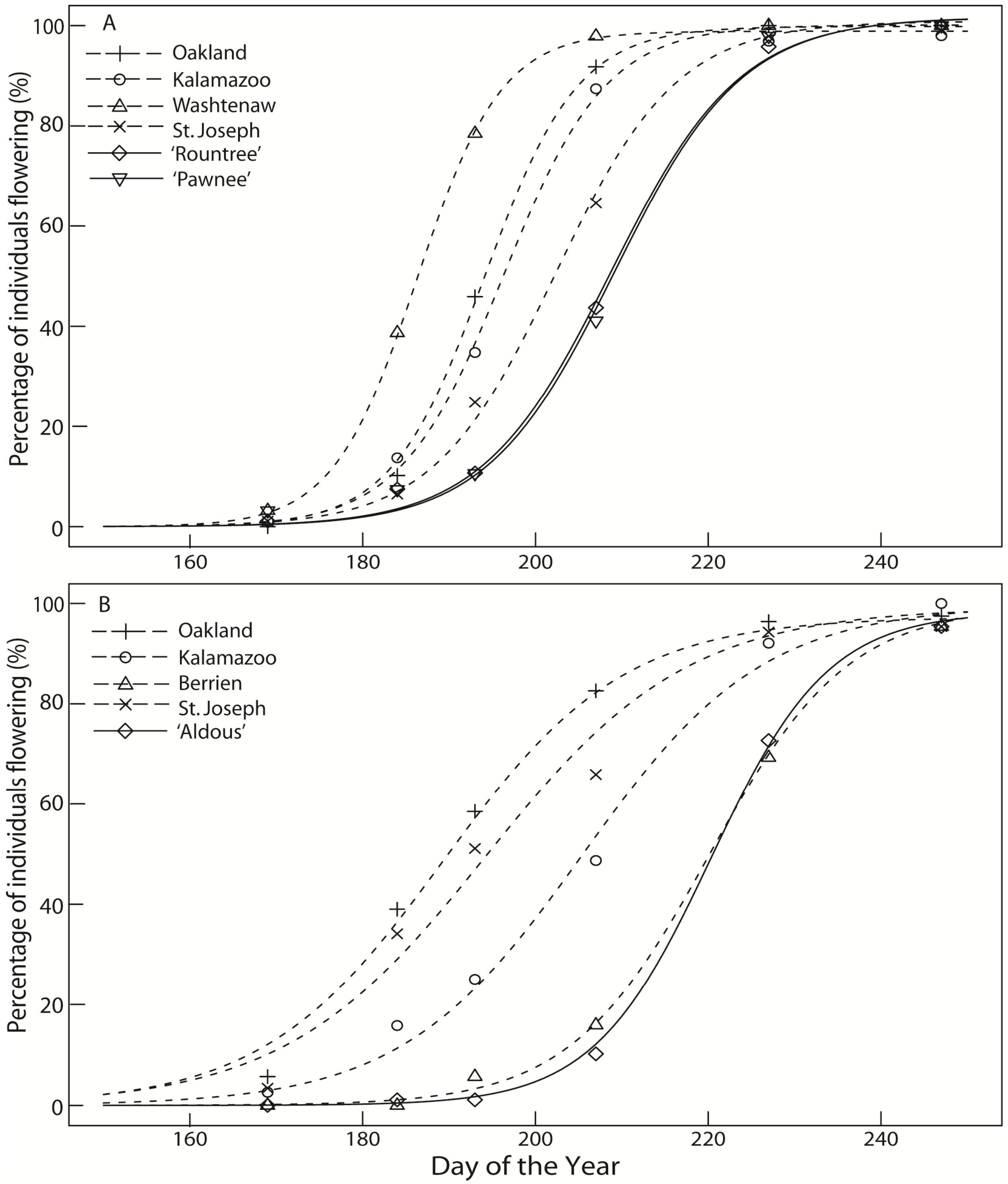

Figure 2.2. Flowering phenology of *A. gerardii* (A) and *S. scoparium* (B) from Yr 2 data. Logistic curves were used to fit the data. Dashed lines are local Michigan populations; solid lines are non-local populations.

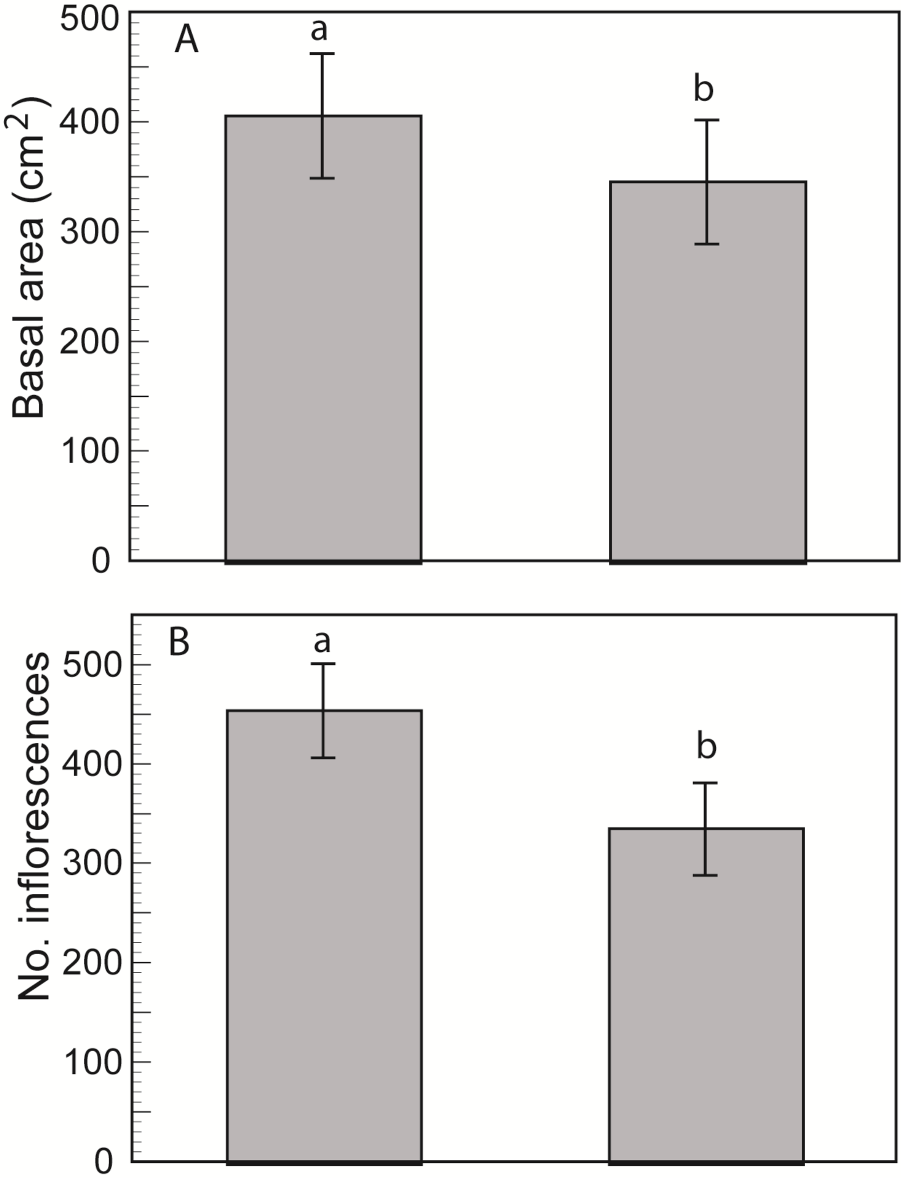

Fig. 2.3. Influence of fertilization on (A) basal area and (B) inflorescence production among *A. gerardii* populations grown in sandy soil plots.

Root fraction

Both propagated and wild-collected populations of *A. gerardii* exhibited similar belowground investment when grown in the Mason sandy soil. Wild-collected populations, propagated populations, and cultivars had similar belowground biomass ($F_{2,7}$ = 0.66, P = 0.55) and root: shoot ratios ($F_{2,7}$ = 2.2, P = 0.18). However, the two intensively selected cultivars (‘Pawnee’ and ‘Rountree’) had somewhat smaller root: shoot ratios compared to all other seed sources (including wild-collected and other propagated sources) ($F_{1,8}$ = 3.75, P = 0.09) (Fig. 2.4A).

The results for aboveground biomass paralleled those from the sandy soil plots in the original common garden experiment; all populations, both propagated and wild-collected, had similar aboveground productivity on the marginal soil ($F_{9,68}$ = 1.48, P = 0.17). This included the non-local, wild-collected population Konza, indicating that geographic origin did not have a strong effect in this case.

Common garden survivorship

Survivorship of *A. gerardii* plants was high in the common gardens. In the initial multi-soil experiment, only two individuals had died by October of Yr 2 (0.3% mortality among all *A. gerardii*). By Yr 4, only two additional individuals had died (overall mortality rate of 0.7%). In the root fraction experiment, two individuals died during the growing season (2.5% mortality).

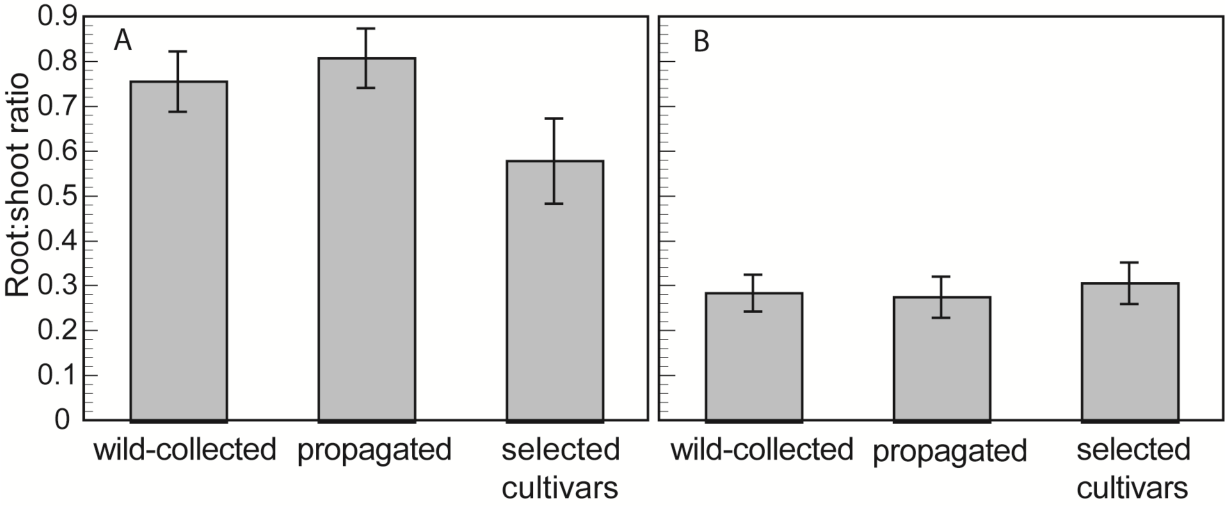

Fig. 2.4. Ratio of root to shoot biomass from (A) *A. gerardii* and (B) *S. scoparium* grown for one season in Mason sandy soil. Population types within a species did not differ (P > 0.05).

Small seedling experiment

When grown from smaller seedlings in a competitive setting, differences between propagated and wild-collected populations were less evident than in the common garden experiment. Plots with only propagated populations of *A. gerardii* produced similar biomass to plots with only wild-collected populations and to mixed plots ($F_{2,7} = 0.31$, $P = 0.74$).

Propagated populations of *A. gerardii* were not stronger competitors than wild-collected populations. If propagated populations had out-competed others, we would expect mixed plots (with both population types) to have lower evenness than plots with only wild-collected populations. However, evenness among *A. gerardii* populations was similar across treatments ($F_{2,7} = 0.58$, $P = 0.59$). In addition, *C. lanceolata* attained similar biomass in all three plot types ($F_{2,7} = 0.11$, $P = 0.89$), indicating that competition with propagated *A. gerardii* populations did not negatively affect this forb's growth.

However, geographic origin did influence population performance in this setting. Local populations had higher survival rates over the 17-month course of the experiment ($F_{1,36} = 30.8$, $P < 0.0001$). This was mostly due to higher mortality among non-local populations in the first 2 months of the experiment ($F_{1,36} = 25.1$, $P < 0.0001$) (Fig. 2.5). Overall, mortality among populations was greater in these early months (32.8% mortality) than months 3-17 (15.4% mortality) ($t = 3.6$, $df = 7$, $P = 0.008$). In particular, plants from the non-local, wild-collected Konza population had very high mortality in the first 2 months (55.3%) when environmental stresses on the small seedlings were likely to be the main cause of mortality. Later in the experiment, when competition was expected to be stronger, differences in mortality were not

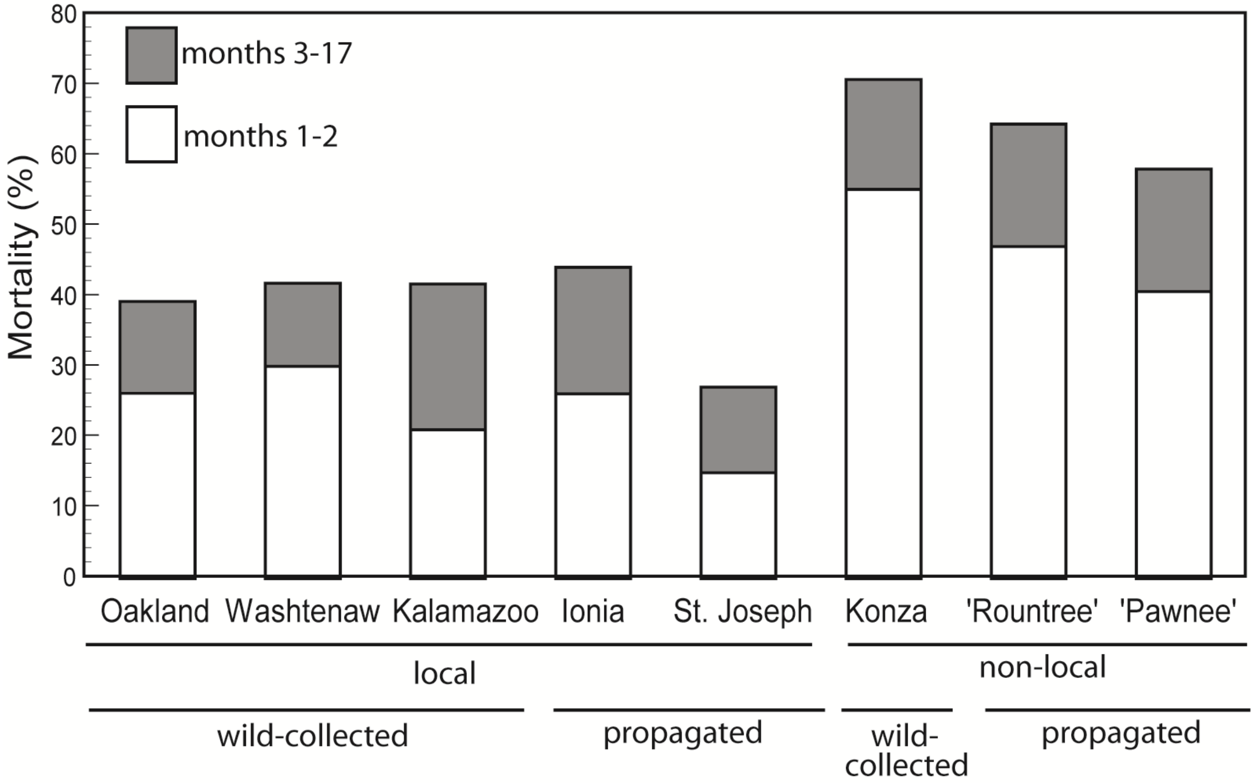

Fig. 2.5. *Andropogon gerardii* population mortality rates when grown in the small seeding experiment.

evident ($F_{1,36} = 0.2$, $P = 0.66$). Thus, our hypothesis that propagated populations would have greater survivorship was not supported.

SCHIZACHYRIUM SCOPARIUM

Vegetative traits

The most striking trend in *S. scoparium* was greatly increased productivity on the Mason sandy soil plot relative to all others (Fig. 2.6A), indicated by a significant interaction between location and soil (Table A2.2). Biomass in the Mason sandy soil was ~250% greater than in other plots in Yr 2 ($F_{1,74} = 65.6$, $P < 0.0001$). Due to the strong differences between the two sandy soil plots, analyses where both sandy soils were grouped together were unable to identify soil type effects (Table A2.2).

Effects of cultivation were less evident in *S. scoparium* than in *A. gerardii*. Contrary to expectations, when categorized as propagated or wild-collected sources, both population types of *S. scoparium* were similar in biomass, height, and basal area (Table A2.2). However, differences in growth traits were evident at the population level (Table A2.2). Both the non-local cultivar (‘Aldous’) and a local, wild-collected source (Berrien) were the largest across soil types. ‘Aldous’ plants were tallest in Yr 1 ($P < 0.001$), but Berrien plants greater basal area in Yrs 1, 2, and 4 ($P < 0.001$). In Yr 4, ‘Aldous’ and Berrien attained similar biomass to each other ($P = 0.95$), but both attained greater biomass than all other populations ($P < 0.05$).

Plant biomass in Yr 2 was highly predictive of biomass production in Yr 4 ($R^2 = 0.44$, $P < 0.001$). All populations exhibited similar ratios of Yr 4 to Yr 1 biomass ($F_{1,4} = 0.36$, $P = 0.84$).

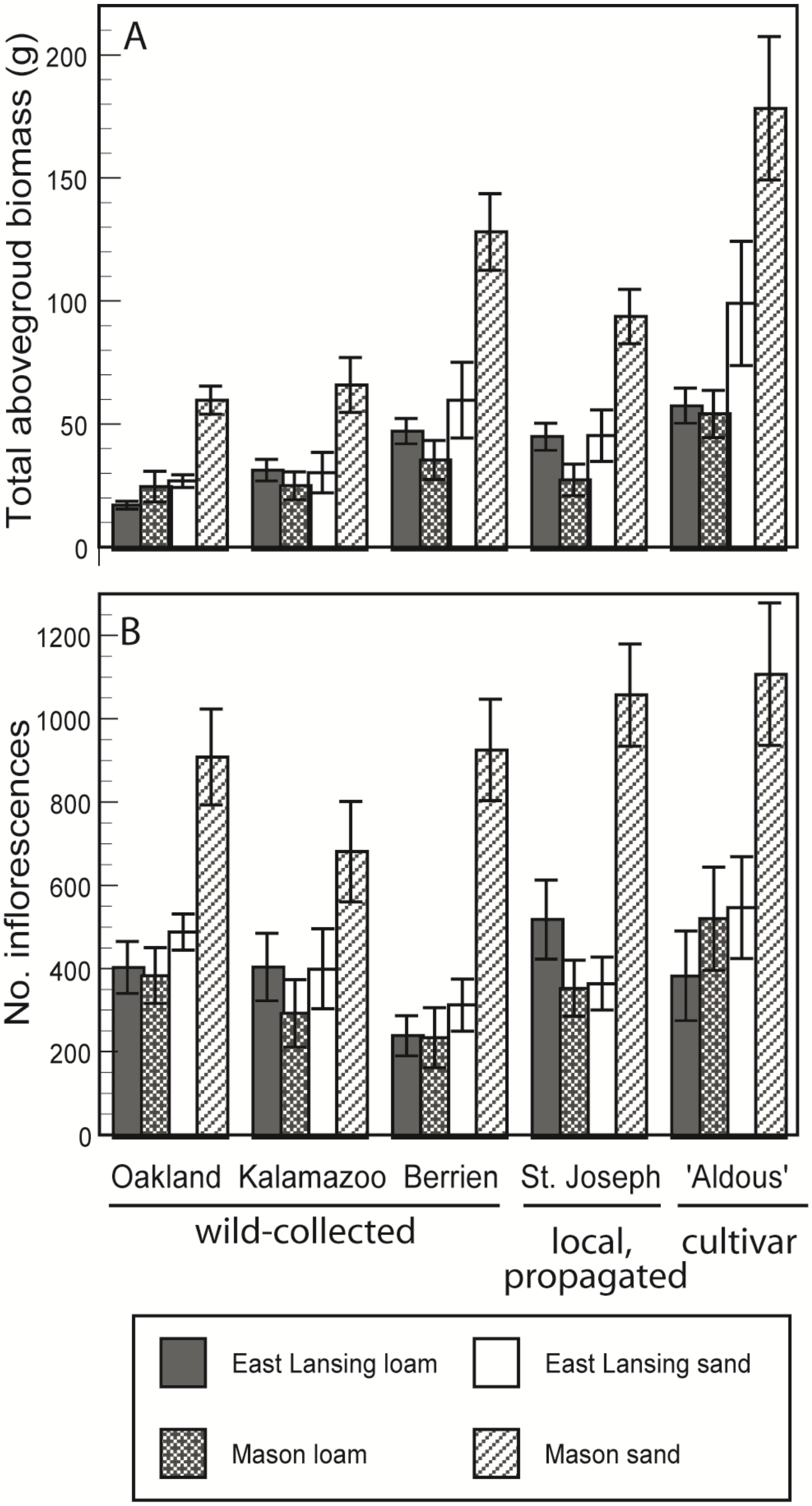

Fig. 2.6. The effect of soil type and population on *S. scoparium* aboveground biomass (A) and inflorescence production on 4 Sept. (B) in Yr 2. Error bars represent standard errors. Both biomass and inflorescence number were greater on the Mason sand than all other plots ($P < 0.05$).

Reproductive traits

Reproductive investment in *S. scoparium* was also markedly greater in the Mason sandy soil than in all other plots (Fig. 2.6B). Across all populations, inflorescence production on the Mason sandy soil was ~240% greater than on other plots in Yr 2 ($F_{1,4} = 66.7$, $P < 0.001$). Population also influenced reproductive investment in Yrs 1 and 2 (Table A2.2). In Yr 1, plants of the non-local cultivar 'Aldous' had more inflorescences than plants from any of the wild-collected populations ($P < 0.05$), but inflorescence production was similar in the local, propagated population, St. Joseph and cultivar 'Aldous' ($P = 0.47$). However, differences in inflorescence production decreased in Yr 2, and 'Aldous' had more inflorescences than the wild-collected population Berrien ($P = 0.02$) but did not differ from any others ($P > 0.05$). Individual seed mass in Yr 2 was the greatest in the wild-collected Berrien population ($P < 0.05$), followed by the cultivar 'Aldous'.

Phenology

Spring phenology in *S. scoparium* showed the same pattern as in *A. gerardii*. Tillers emerged earlier on sandy soils than on loamy soils ($\chi^2 = 25.23$, $df = 1$, $P < 0.001$). Emergence was unaffected by either the propagated/wild-collected dichotomy ($\chi^2 = 0.64$, $df = 1$, $P = 0.42$) or by geographic origin (local versus non-local) ($\chi^2 = 0.10$, $df = 1$, $P = 0.75$).

Flowering phenology differed among source populations (Fig. 2.2B). Overall, local plants were more likely to have inflorescences early in the season (June and July) than non-local plants ($\chi^2 = 4.61$, $df = 1$, $P = 0.03$). However, the propagated, non-local population 'Aldous' and the wild-collected, local population Berrien were the last populations to begin flowering.

Fertilization response

Fertilization did not measurably affect the growth of *S. scoparium* plants; fertilized and unfertilized *S. scoparium* plants in sandy soils were similar in height ($F_{1,33} = 2.92$, $P = 0.1$) and basal area ($F_{1,33} = 0.001$, $P = 0.98$). In addition, no delayed effects of fertilization were evident in Yr 4 ($P > 0.05$).

Root fraction

Comparisons of root fraction of plants grown in the Mason sandy soil were similar to results from *A. gerardii*; neither aboveground biomass ($F_{7,56} = 0.94$, $P = 0.48$), belowground biomass ($F_{7,56} = 1.15$, $P = 0.35$), nor root: shoot ratios ($F_{7,56} = 0.4$, $P = 0.90$) varied among populations or among population types ($P > 0.05$) (Fig. 2.4B). This included the non-local, wild-collected Konza population, which produced similar biomass to all other populations ($P > 0.05$).

Survivorship

Among *S. scoparium* populations, overall mortality rate in the multi-soil common garden experiment was 7.2% by October of Yr 2; by spring of Yr 4, it had reached 12.3%. Population type did not influence mortality as had been expected; wild-collected and propagated populations experienced similar mortality rates ($\chi^2 = 0.4$, $df = 1$, $P = 0.54$). Survivorship among *S. scoparium* plants was lower (87.7%) than among *A. gerardii* (99.3%) (Yr 4: $\chi^2 = 67.5$, $df = 1$, $P < 0.0001$) in the main experiment. However, plants on loamy soils were more than twice as likely to die (15.6% mortality) compared to plants on sandy soils (6.8% mortality) ($\chi^2 = 8.4$, $df =$

1, *P* = 0.004). In the shorter-term root fraction experiment, all of the *S. scoparium* plants survived to harvest.

ENVIRONMENTAL VARIABLES AND GOWER SIMILARITY INDEX

Soil characteristics of common garden plots differed from those of seed collection and propagation sites in Michigan, as demonstrated by principal component analysis (Fig. 2.7). The Mason loamy soil plot was markedly different from all other locations, in part due to very high phosphorous content. Three soil characteristics—organic matter, cation exchange capacity, and total nitrogen—were all highly related with negative loading values on PC1.

In the analysis using only common garden soils, growth and reproductive investment in *A. gerardii* were related to PC1, PC2, and PC3, with PC3 explaining the most variation in these traits (Table 2.3). The response of *S. scoparium* was similar, with the exception that inflorescence production was not significantly related to PC2 (Table 2.3). PC1 is most strongly influenced by total nitrogen, organic matter, CEC, and phosphorous; PC2, by nitrate and ammonium. PC3 is mostly strongly influenced by pH, cation exchange capacity (CEC), and phosphorous, suggesting that these were key factors in plant performance. However, the nature of the relationship differed between the two species (Table 2.3). For example, *A. gerardii* was most productive on soils with intermediate values for PC3, whereas *S. scoparium* was most productive at low and high values for PC3 (Fig. 2.8).

Andropogon gerardii plants from origins with higher latitudes tended to flower earlier than plants from lower latitudes (linear regression, R^2 = 0.61, *P* = 0.07) (Fig. 2.9), but this was not the case for *S. scoparium* (R^2 = 0.43, *P* = 0.23).

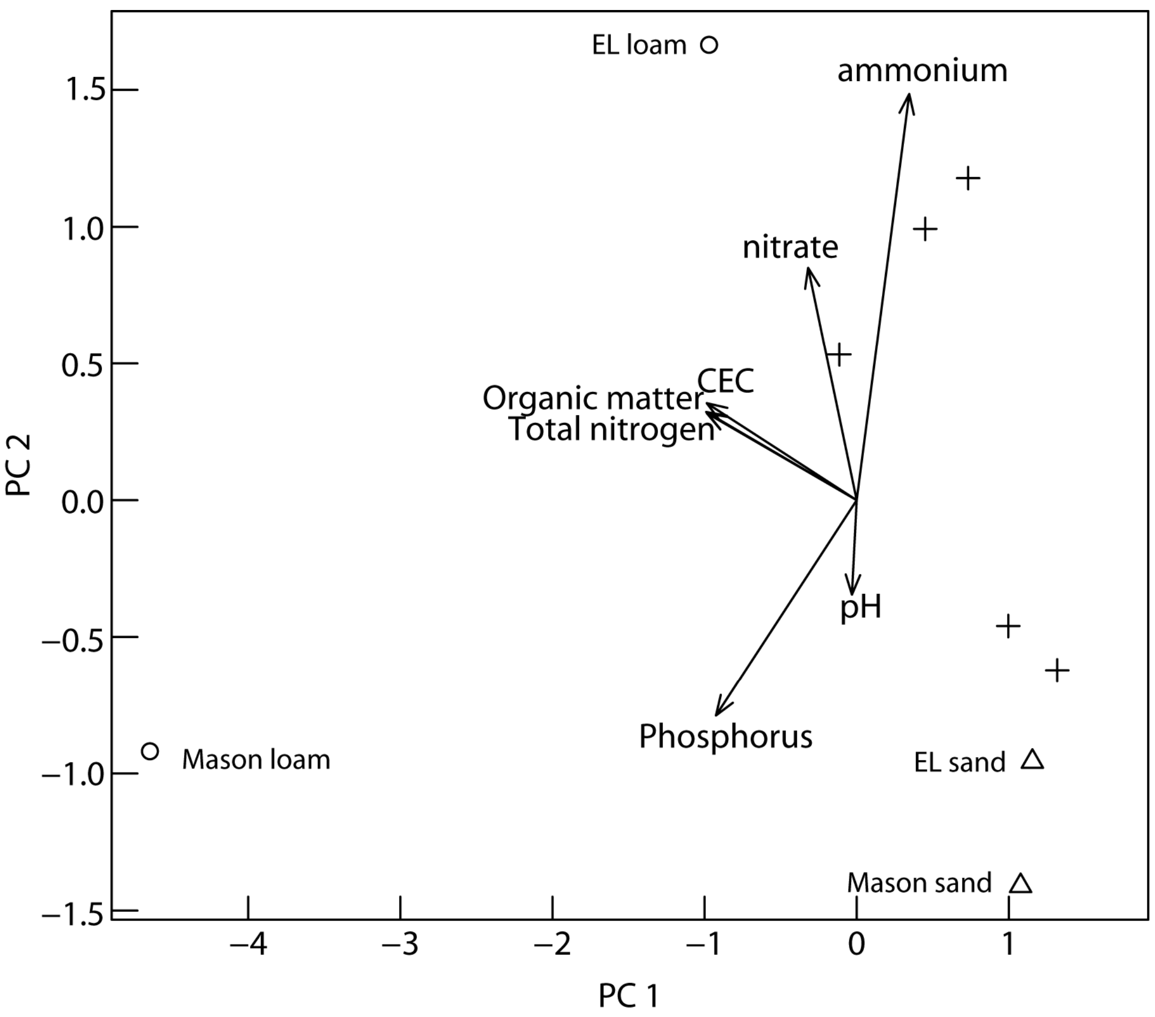

Fig. 2.7. Distribution of common garden plots and Michigan population sources based on principal component analysis of soil characteristics showing that collection site soils differed from both loamy and sandy common garden soils. Arrows represent relative loadings of soil characteristics on PC1 and PC2. (○ = loamy plots, Δ = sandy plots, + = sites of local Michigan populations)

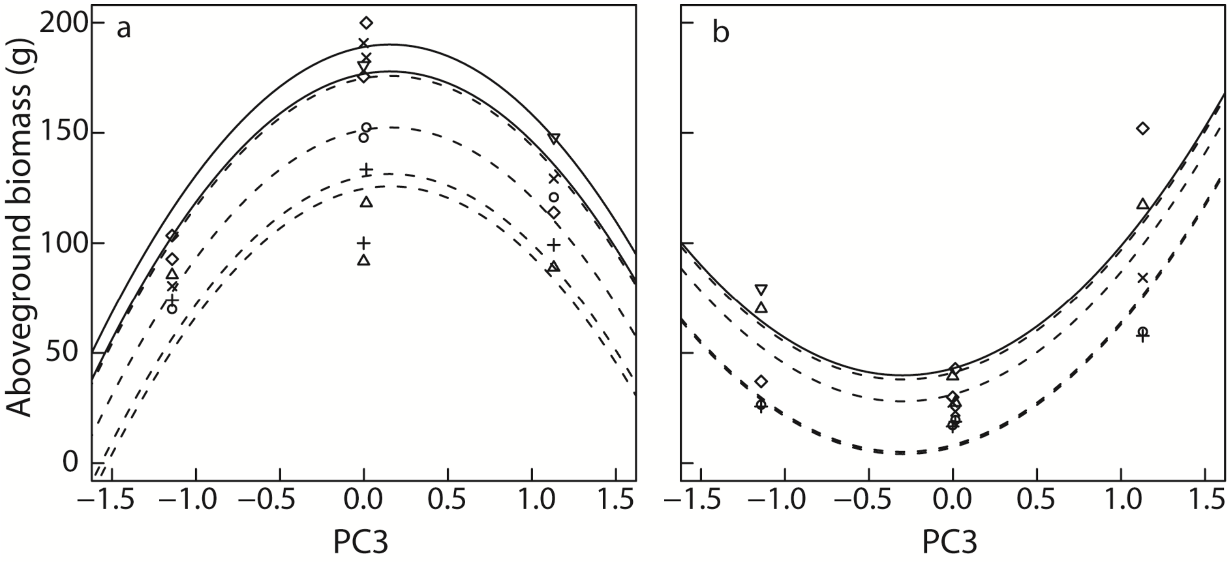

Fig. 2.8. Relationship between principal component 3 for common garden plot soil characteristics and biomass production in (A) *A. gerardii* and (B) *S. scoparium*. Symbols represent populations (+ = Oakland, ○ = Kalamazoo, △ = (A)Washtenaw/(B)Berrien, x = St. Joseph, ◊ = (A)'Rountree'/(B)'Aldous', ▽ = 'Pawnee').

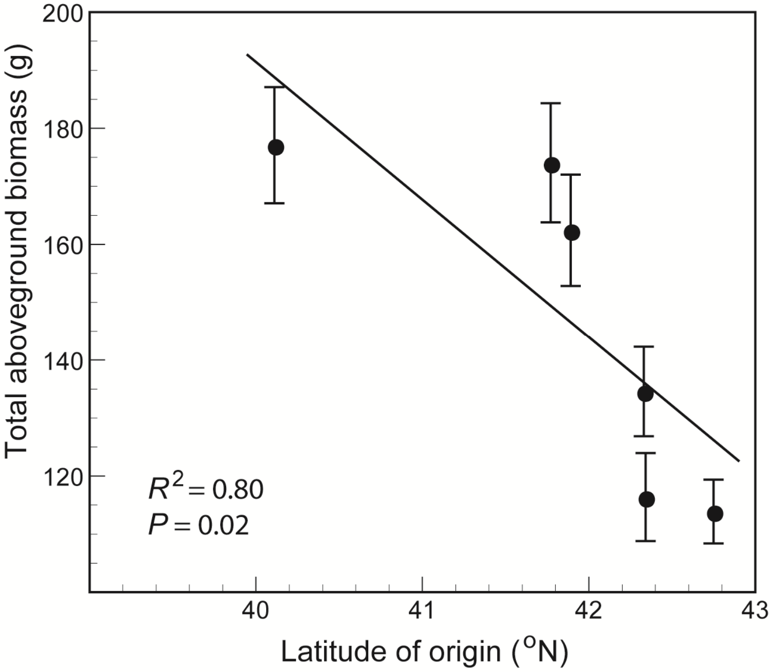

Fig. 2.9. Influence of population latitude of origin on average population biomass production in *A. gerardii*.

Table 2.3. Results of ANCOVA testing associations between population performance and principle component values for soil characteristics of common garden plots. Model R^2 values are for full ANCOVA models, including population as a categorical variable.

		Aboveground biomass					No. inflorescences				
		Linear term		Quadratic term		Model	Linear term		Quadratic term		Model
Species	Principle component	Estimate	*P*	Estimate	*P*	R^2	Estimate	*P*	Estimate	*P*	R^2
A. gerardii	PC1	-0.6±0.1	**0.0001**	**-**	-	0.72	-1.9±0.3	<**0.0001**	-0.7±0.2	**0.0003**	0.78
	PC2	0.3±0.3	0.2	-	-	0.37	1.1±0.3	**0.007**	**-**	-	0.43
	PC3	0.7±0.2	**0.007**	-1.9±0.3	<**0.0001**	0.85	0.9±0.3	**0.006**	-3.3±0.4	<**0.0001**	0.87
S. scoparium	PC1	1.3±0.3	**0.0008**	0.5±0.2	**0.02**	0.69	1.6±0.6	**0.02**	-	-	0.41
	PC2	-0.6±0.3	*0.09*	-	-	0.37	-1.1±0.9	0.26	-	-	0.19
	PC3	1.3±0.3	**0.0007**	2.4±0.4	<**0.0001**	0.87	4.6±0.5	<**0.0001**	5.2±0.65	<**0.0001**	0.92

If environmental similarity between population source and common garden sites were a good predictor of population performance, the Gower index would be expected to be significantly and negatively related to measures of plant growth: more similarity (i.e., lower Gower index) would lead to greater biomass or inflorescence production. However, *A. gerardii* performance measures were positively related to Gower indices for soils (Table 2.4). This suggests that home sites for this species are not optimal for growth and reproductive investment. On the other hand, the relationship between *S. scoparium* performance and soil characteristics was consistent with our hypothesis; biomass production was negatively related to Gower indices for soils (Fig. 2.10), indicating that growth was greater when plants were grown in soils most similar to home sites. A similar pattern was also found for reproductive investment of *S. scoparium* (Table 2.4).

Overall, Gower distances indicated that soil characteristics from seed collection locations were more similar to those of sandy common garden plots (especially the East Lansing sand) than to those of loamy plots (Table A2.3). However, *A. gerardii* plants grew larger in loamy plots, and *S. scoparium* plants generally grew larger in the Mason sand than the East Lansing sand.

Discussion

PLASTICITY IN RESPONSE TO SOIL RESOURCES

Propagated populations of *A. gerardii* demonstrated greater phenotypic plasticity in response to growth in fertile soils than did wild-collected populations. To some extent, plasticity in plant traits can result directly from limitations to plant growth. However, plasticity may also allow some species or genotypes either to minimize negative fitness effects due to low resource

Table 2.4. Results of correlation analysis testing associations between population performance and Gower indices calculated using assessments of soil characteristics. If similarity between home sites and common gardens increases performance, significant negative relationships are expected between Gower indices and performance measures.

Species	Response variable	*r*	*P*
A. gerardii	Aboveground biomass	0.41	0.11
	No. inflorescences	0.41	0.12
S. scoparium	Aboveground biomass	**-0.60**	**0.01**
	No. inflorescences	*-0.47*	*0.06*

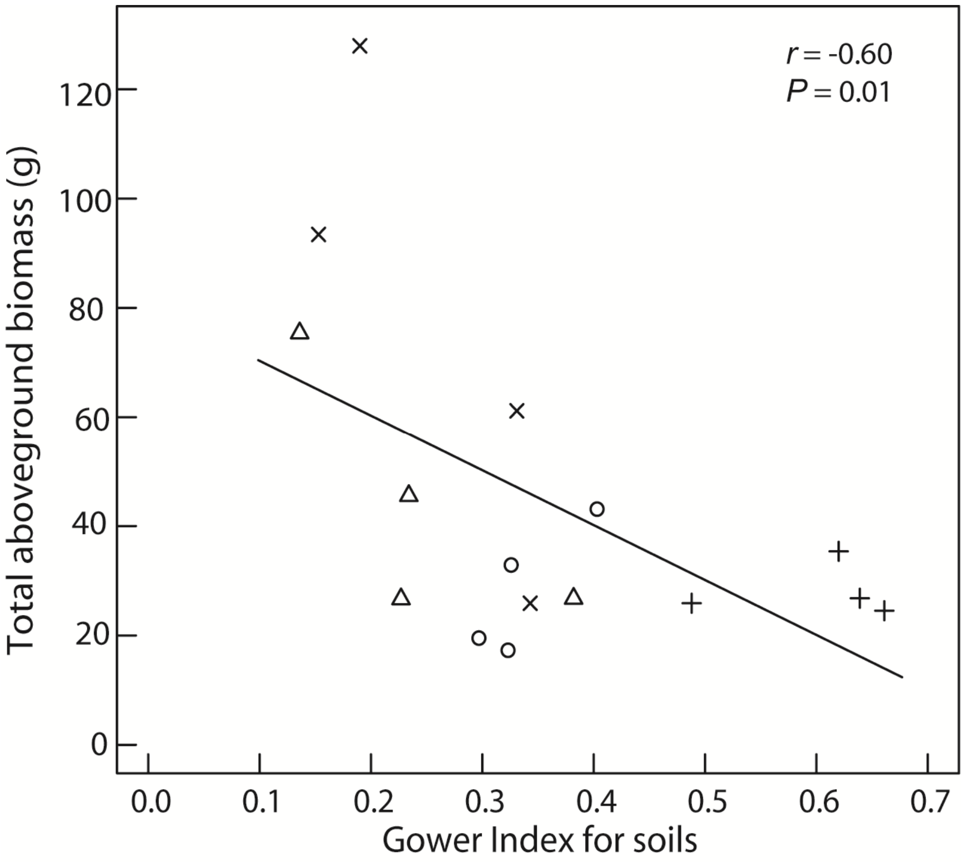

Fig. 2.10. Relationship between Gower index for soil characteristic similarities and biomass production in populations of *S. scoparium*. The negative association indicates that S. *scoparium* populations produced more biomass when grown in soils most similar to their home sites. Symbols represent the four common garden plots (○ = EL loam, + = Mason loam, Δ = EL sand, x = Mason sand).

availability (Elberse et al. 2003, Sultan 2003) or to maximize acquisition of available resources (Sultan 2000). In our study, both propagated and wild-collected populations of *A. gerardii* performed similarly under stressful conditions of droughty, nutrient-poor soil, but propagated populations responded to fertile soils by increasing both vegetative and reproductive investment more than wild-collected populations.

Selection during propagation in *A. gerardii*, therefore, appears to have selected for a different norm of reaction than is found in wild populations, such that productivity in propagated varieties is greatly increased in response to resource availability. This may be related to the conditions of propagation and harvest methods. Fertile fields may be chosen and water and nutrients may be subsidized to increase seed yields in propagation fields. Those plants best able to utilize additional water and nutrient resources are likely to be the largest, tallest and therefore are most likely to have their seed harvested.

Schizachyrium scoparium, on the other hand, showed a plastic response in the opposite direction; plants were more productive on one of the marginal soils. In addition, this plastic response was not strongly associated with cultivation history.

In crop development, plasticity is sometimes perceived as a problem because genotype by environment interactions can lead to reduced yields under some conditions (Kang 1998), but genotype by environment interactions have been responsible for yield gains among many crops under high-input agricultural conditions (Simmonds 1981). However, crop development for low-input systems or marginal lands may require different practices such as low-input selection trials (Simmonds 1991) or selection for trait stability across environments (Simmonds 1981).

Selection for trait stability might be more feasible than developing cultivars able to deal with specific limiting factors, particularly because limiting resources may vary among marginal sites. Our study demonstrates that “marginal” and “fertile” can be an over-simplification; *S. scoparium* was highly productive in one sandy soil and much less so in another. One possible explanation is that *S. scoparium* experienced negative interactions in moist soils (potentially pathogen pressure), which reduced growth on fertile soils, but that nutrient levels were too low in the East Lansing sandy soil to support the productivity seen in the Mason sandy soil.

GROWING CONDITIONS AND PROPAGATION PRACTICES

Propagation exposes populations to a range of selective pressures that differ from those in nature. For profitable seed production, commercial seed producers situate propagation fields on fertile soils, and seed yields may be increased through fertilizer additions (Cornelius 1950). Environmental conditions in propagation fields are usually more homogeneous than in nature.

Seed harvest methods may also act as a filter on plant traits and reduce genetic variability (Knapp and Dyer 1998). Mechanical harvest is likely to gather seed from plants taller than a given threshold, and thus, shorter or less mature plants may contribute few seeds to the harvest. The strength of this filter may depend on whether or not seed is harvested at multiple times.

In high fertility soils, those plants best adapted to higher resource availability may dominate and produce the most seed, and so practices of fertilization and irrigation could lead to selection for genotypes that respond strongly to resource availability. This parallels population dynamics in nature where population differentiation occurs in response to different environmental conditions (Pickett 1976). The cultivars in our study have likely undergone many rounds of selection as new propagation fields have been planted from the seeds of previous fields.

However, St. Joseph *A. gerardii*, which was only recently brought into cultivation, responded to the fertile soil in a manner similar to cultivars propagated for decades. Soil nutrients differed little between propagation fields for St. Joseph populations and sites of wild-collected populations (Table 2.2). Thus, it may be that irrigation, although infrequent, exerted selective pressures on *A. gerardii*. Another contributing factor may be intraspecific competition in production fields, such that genotypes able to grow rapidly out-compete other genotypes during plant establishment, and larger plants also may contribute more heavily to seed harvests. Another factor may be heterosis or hybrid vigor, which can occur as a result of crosses between unrelated individuals (Vergeer et al. 2004), because St. Joseph propagation fields were established with seed from multiple sites. However, why heterosis would be more evident under fertile conditions is unclear.

GEOGRAPHIC AND ENVIRONMENTAL DISTANCES

In our study, the effects of geographic distance were mainly evident in population differences in flowering phenology, with populations from higher latitudes flowering earlier than populations of more southern origin. This trend towards earlier flowering among northern populations of *A. gerardii* and *S. scoparium* has also been found in common garden studies in Texas, Nebraska, and Minnesota (McMillan 1959, 1965, Olson 1986). Based on these findings, Olson (1986) supports the guideline that varieties not be planted more than 483 km (300 miles) northward of their origin in the U.S. prairie pothole region (Duebbert et al. 1981). The population from the greatest geographic distance, the cultivar ‘Aldous,’ originated from an area approximately 400 km south of the common garden sites, so the populations used in this study fall within the suggested range. Although not seen in this study, more extreme delays in flower phenology could lead to a failure to set seed. This could reduce natural re-seeding in restoration and reduced profitability in a propagation field. However, delayed flowering can be a benefit to biofuel production; in *P. virgatum*, biomass accumulation was greater in populations with later heading dates (Berdahl et al. 2005).

When plants of either *A. gerardii* or *S. scoparium* were grown individually, local populations did not show any advantages compared to non-local populations that would have indicated local adaptation; non-local plants grew equally well or better than most local plants based on measures of biomass production and reproductive investment. In contrast, in the small seeding experiment, survival of plants from non-local *A. gerardii* populations were lower than that of plants from local populations. Because most mortality was in early in the experiment before competition intensified, differential survival rates may be due to more intense selection pressure against non-local genotypes at the seedling stage. This is consistent with other work suggesting that lack of local adaptation may be "hidden" under some conditions and revealed when stresses are more intense (Knapp and Dyer 1998). In this study, the stresses encountered by small seedlings may have revealed such a difference while growth on marginal soils in a noncompetitive environment did not. The non-local, wild-collected Konza population from Kansas performed equally well when grown without competition for one season in sandy soil, but this same population had lower survival and reduced biomass compared to other populations in the competition experiment.

SPECIES DIFFERENCES

Our study suggests that species can respond differently to selection pressure during propagation, even when the species are often found as co-dominants, like *A. gerardii* and *S.*

scoparium. Although the species commonly co-occur, *A. gerardii* dominates in wetter soils of lowland areas in both tallgrass and mixed grass prairie, and *S. scoparium* dominates in drier, upland habitat in tallgrass prairie (Weaver 1968) and on slopes with thin soil in mixed grass prairie (Albertson 1937). Although competition with *A. gerardii* can reduce establishment of *S. scoparium* in mesic soils (Howell and Kline 1992), our study suggests that competition is not the only limiting factor for *S. scoparium* in fertile soils because, at least at the Mason location, *S. scoparium* plants grew larger in the sandy soil than in the loamy soil. Adaptation to different environments between the two species may have affected the genetic variability available for selection during cultivation, particularly for traits associated with resource capture in fertile soils.

DOMESTICATING NATURE

Anthropogenic influence on global ecosystems is pervasive (Kareiva et al. 2007). In maximizing one ecosystem product or service, humans often create unintended consequences for other services. Domestication of nature may be obvious in some cases, such as the use of Virginia strawberry, *Fragaria virginiana*, to produce commercial varieties (Fletcher 1917). However, in other cases domestication may be less apparent, such as in areas of tropical forest managed by local farmers to favor desirable tree species (Michon et al. 2007).

In the case of native prairie species, humans have altered gene flow patterns among wildland populations due to the high level of prairie fragmentation (Sieg et al. 1999) and have increased the prevalence of particular genotypes through propagation and widespread distribution of commercial cultivars. To more fully assess the consequences of anthropogenic changes to native species, further work should address how propagated populations respond to more extreme episodic environmental stresses, such as cold snaps or prolonged drought, and to disease pressure. Due to trade-offs between growth and defense (Herms and Mattson 1992, Todesco et al. 2010), selection for fast growth may compromise plant defenses against pathogens (Schrotenboer et al. 2011). Evidence from domesticated food crops indicates that cultivars are often more susceptible to herbivores and pathogens than wild relatives (Rosenthal and Dirzo 1997, Lindig-Cisneros et al. 2002).

Currently, prairie grasses and other “wild” species are being developed for use as biofuel crops (James et al. 2010). As a result, changes to plant traits are likely to be more radical than those seen among current cultivars. The same varieties modified for biofuels may also be used for restoration (as has been the case with forage cultivars), and these varieties may also interact with conspecific remnant populations. Thus, understanding how cultivation affects native species is key to evaluating species and landscape management practices.

MANAGEMENT RECOMMENDATIONS FOR RESTORATION AND BIOFUELS

Ultimately, the choice of appropriate seed sources will depend on project goals. Different populations show variation in traits and performance, and thus, not all seed sources will be appropriate for all projects. Our work suggests that a history of propagation is at least equally important to consider as local or non-local origin when choosing plant material for ecological restoration or biofuel cropping with native species.

Propagated populations of prairie grasses (particularly *A. gerardii*) may provide the benefit of rapid growth on fertile soils when soil conservation or ruderal invasive species are a concern. However, if the conservation of local diversity or of historical landscapes is a restoration priority, wild-collected populations will better meet these needs. For development as biofuel crops, *S. scoparium* is currently well suited for use on marginal lands, whereas *A.*

gerardii cultivar development may require focused breeding to increase plant productivity in marginal soils. Future cultivar modifications could increase the stability of biomass production across environments. Alternatively, specific cultivars could be developed for both marginal and fertile soils. Consideration of potential effects on other landscape components, such as gene flow into remnant populations, may also factor into selection of plant materials used in restorations or biofuel plantings.

CHAPTER 3

MODIFICATION OF NATIVE GRASSES FOR BIOFUEL PRODUCTION MAY INCREASE VIRUS SUSCEPTIBILITY

Abstract

Bioenergy production is driving modifications to native plant species for use as novel biofuel crops. Key aims are to increase crop growth rates and to enhance conversion efficiency by reducing biomass recalcitrance to digestion. However, selection for these biofuel-valuable traits has potential to compromise plant defenses and alter interactions with pests and pathogens. Insect-vectored plant viruses are of particular concern because perennial crops have potential to serve as virus reservoirs that influence regional disease dynamics. In this study, we examined relationships between growth rates and biomass recalcitrance in five switchgrass (*Panicum virgatum*) populations, ranging from near-wildtype to highly selected cultivars, in a common garden trial. We measured biomass accumulation rates and assayed foliage for acid detergent lignin, neutral detergent fiber, *in vitro* neutral detergent fiber digestibility and *in vitro* true dry matter digestibility. We then evaluated relationships between these traits and susceptibility to a widely distributed group of aphid-transmitted Poaceae viruses (Luteoviridae: *Barley and cereal yellow dwarf viruses*, B/CYDVs). Virus infection rates and prevalence were assayed with RT-PCR in the common garden, in greenhouse inoculation trials, and in previously established switchgrass stands across a 300-km transect in Michigan, USA. Aphid host preferences were quantified in a series of arena host choice tests with field-grown foliage. Contrary to expectations, biomass accumulation rates and foliar digestibility were not strongly linked in switchgrass populations we examined, and largely represented two different trait axes. Natural B/CYDV prevalence in established switchgrass stands ranged from 0 - 28%. In experiments, susceptibility varied notably among switchgrass populations and was more strongly predicted by potential biomass accumulation rates than by foliar digestibility; highly selected, productive cultivars were most virus-susceptible and most preferred by aphids. Evaluation and mitigation of virus susceptibility of new biofuel crops is recommended to avert possible unintended consequences of biofuel production on regional pathogen dynamics.

Introduction

At present, the North American biofuel industry relies heavily on ethanol-based fuels produced through maize grain fermentation (de Vries et al. 2010). To broaden the plant materials available for ethanol production, new methods for converting plant lignocellulosic material to ethanol are being developed for deployment in the coming decade (Gomez et al. 2008). Much attention is focused on developing perennial grasses as novel second-generation biofuel crops that could provide multiple environmental advantages, such as increased soil carbon storage, while supporting reliable bioenergy production (Lemus and Lal 2005, Heaton et al. 2008). Switchgrass (*Panicum virgatum* L.) is one of the strongest contenders as a perennial biofuel crop in the United States and is also being considered for use in Canada and Europe (Samson and Omielan 1992, Smeets et al. 2009). Switchgrass is a warm-season native prairie grass from North America and was historically used for forage and erosion control (Vogel 2004).

In modifying switchgrass and other grasses for biofuel production, two key aims are to increase crop productivity (Karp and Shield 2008) and to improve conversion efficiency by reducing biomass recalcitrance to enzymatic and microbial digestion (Himmel et al. 2007). Here we examine the extent of such trait modifications in switchgrass, evident in near-wildtype populations and developed cultivars, and evaluate relationships between these traits and virus susceptibility. Whereas pathogens of maize and other food crops are largely well understood, pathogen interactions with novel bioenergy crops have been less well explored and deserve significant attention. Unless mitigated, pathogens have the potential to depress feedstock yields and even spill over into other crops in the region (Spencer and Raghu 2009, Agindotan et al. 2010). Previous anthropogenic changes to plant traits and distributions have driven pathogen emergence and development of disease outbreaks (Kennedy and Barbour 1992, Webster et al. 2007, Jones 2009), so potential consequences of biofuel crop deployment for pathogen dynamics merit investigation.

A major aim of increasing the productivity of novel biofuel crops, such as perennial grasses, is to make their expected profitability competitive with first-generation crops, such as maize (James et al. 2010). However, plant allocation theory predicts that increases in growth rates often come at the cost of reductions in defense (Herms and Mattson 1992); as a result, selection for increased productivity may inadvertently increase plant disease susceptibility of these new crops. Likewise, efforts to reduce lignin in crop species have raised concern about the potential for increased pest and pathogen pressure (Li et al. 2008).

Although links between reduced lignin and increased herbivore pressure remain unclear (Pedersen et al. 2005), some evidence suggests that chewing herbivores (mammals or insects) may sometimes prefer or benefit from low lignin plants. For example, brown midrib (BMR) lines of sorghum-sudangrass and pearl millet, which have reduced lignin, were preferred over wildtype varieties by grazing lambs (Cherney et al. 1990, Li et al. 2008). The effects of lignin on insect herbivores are complex. For example, across a broad spectrum of sorghum lines, high lignin concentration was associated with resistance to fall armyworms, as measured by duration of larval development. However, among the most resistant sorghum lines, larvae developed more rapidly on lines with the highest lignin levels (Diawara et al. 1991). In more recent work with transgenic aspen and silver birch lines with altered lignin content, differences in growth rates and preferences among lepidopterans and coleopterans were not clearly attributable to lignin (Tiimonen et al. 2005, Brodeur-Campbell et al. 2006). The complexity of these interactions may be explained in part by plant allocation to other defensive pathways, such as production of phenolic compounds (Brodeur-Campbell et al. 2006), in addition to or in place of lignin biosynthetic pathways. The response of sucking insects, like aphids and thrips, to changes in lignin in biofuel feedstocks has not been previously addressed.

Numerous microbes and insect pests have potential to cause damage in biofuel crops. Highly visible pathogens, such as fungal diseases, are among the first to have been identified as possible problems in switchgrass (Gustafson et al. 2003, Crouch et al. 2009). Less visible pathogens, such as viruses, may be more easily overlooked in field trials and thus merit deliberate investigation. Viruses have likely influenced crops since the dawn of early agriculture (Gibbs et al. 2008, Gibbs et al. 2010), and their potential interactions with novel biofuel crops cannot be ignored.

Our long-term goal is to assess how trait changes associated with bioenergy crop development could alter ecological interactions between Poaceae species and Poaceae-infecting viruses at multiple scales. In the study reported here, we examine the influence of human selection pressure on susceptibility of switchgrass populations to infection by *Barley and cereal yellow dwarf viruses* (B/CYDVs), a key group of globally important pathogens. Our specific objectives are (1) to examine the influence of anthropogenic selection pressures on key biofuel-valued traits (biomass recalcitrance and growth rates) in existing switchgrass populations; (2) to quantify the susceptibility of these same populations to virus infection; and (3) to investigate the nature of any relationships between biofuel-valued traits and virus susceptibility.

Methods

COMMON GARDEN EXPERIMENT

For this study, we conducted a suite of field and greenhouse experiments. To evaluate differences in growth traits among switchgrass populations, we first established a common garden experiment in the field at Michigan State University's Horticulture Teaching and Research Center in East Lansing, MI (42.7ºN 84.5ºW). Soils at the site are loamy alfisols and mollisols (Aquic Glossudalfs, Typic Endoaquolls, and Typic Argiaquolls). East Lansing averages 796 mm of precipitation per year and has an average maximum daily temperature of 13.9ºC and average minimum daily temperature of 2.7ºC (Michigan State Climatologist's Office, http://climate.geo.msu.edu).

To compare effects of different selection pressures on switchgrass traits and performance, we chose five commercially available populations, either native Michigan genotypes or cultivars suitable for use in Michigan (Michigan Department of Natural Resources), which represented a spectrum from near-wildtype populations (Michigan Wildflower Farm, Southlow) to cultivars developed for use as forage grasses ('Nebraska 28', 'Blackwell', 'Trailblazer') (Table 3.1) . 'Trailblazer' is the most intensively selected cultivar, having been selected initially for vigor and then for tissue digestibility (Vogel and Moore 1993, Vogel and Pedersen 1993). Second-generation switchgrass cultivars currently in development for biofuel purposes are likely to further extend the trajectory of this spectrum towards greater productivity and digestibility.

Table 3.1. Characteristics of switchgrass populations included in common garden and aphid preference experiments.

Experiment	Population	Level of selection	Geographic origin	Release date	Population type
Common garden	Michigan Wildflower Farm (MWF)	Near-wildtype	Michigan	–	Near-wildtype. Propagated for native restoration.
	Southlow	Near-wildtype	Lower Michigan	2001	Registered germplasm. Multi-origin G1 seed assembled from 11 native G0 stands[1]
	Nebraska 28	Moderately selected	Nebraska	1949	Registered variety.
	Blackwell	Moderately selected	Oklahoma	1944	Registered variety. Single-plant origin.
	Trailblazer	Intensively selected	Kansas and Nebraska	1984	Registered variety.
Aphid preference tests	Dacotah	Moderately selected	North Dakota	1989	Registered variety.
	Cave-in-Rock	Moderately selected	Illinois	1973	Registered variety.
	Pathfinder	Moderately selected	Kansas and Nebraska	1967	Registered variety.
	Shawnee	Intensively selected	Illinois	1995	Registered variety. Cave-in-Rock used as base population[2]

Table 3.1 (cont'd)

Population	Characteristics selected for	Other notable characteristics	Disease characteristics
Michigan Wildflower Farm (MWF)	–	–	–
Southlow	Native diversity	Mixed ploidy[3]	–
Nebraska 28	–	Leafy, medium height, early maturing[4] octoploid[5,6], upland[6]	Rust susceptible[4]
Blackwell	–	Leafy, medium height, good forage production[4], octoploid[5,7], upland[6]	Good stem rust resistance[4]
Trailblazer	Forage quality, high in vitro dry matter digestibility[8]	Leafy, good forage production, late maturing[4], octoploid[7,9], upland[6]	Rust susceptible in a field study[10]
Dacotah	Leafiness, vigor, seed production, winter hardiness[4]	Short height, drought tolerance[4], tetraploid[11]	Leaf spot susceptible in a field study[12]
Cave-in-Rock	–	Vigorous, high seed yield, resistant to lodging[4], octoploid[7,13]	Resistant to leaf spot[4]
Pathfinder	–	Vigorous, leafy, late maturing[4], octoploid[5,7]	Rust resistant[4]
Shawnee	High in vitro dry matter digestibility, yield, leafiness[2]	Octoploid[2]	Disease resistant compared to Cave-in-Rock[2]

-, data not applicable or available

[1] Durling et al. 2008

[2] Vogel et al. 1996

[3] D. Schemske, unpublished results

[4] Alderson and Sharp 1995

[5] Riley and Vogel 1982

[6] Vogel 2004

[7] Hopkins et al. 1996

[8] Vogel et al. 1981

[9] Vogel et al. 1991

[10] Redfearn et al. 1999

[11] Narasimhamoorthy et al. 2008

[12] Krupinsky et al. 2004

[13] Wullschleger et al. 1996

Switchgrass individuals were started from seed in a virus-free greenhouse in April 2008. They were planted into the field as plugs (set 1 m apart) in June 2008 in a completely randomized design (*n* = 9–10 per population after minimal initial mortality). Plants were open-grown with minimal competition from neighbors; weeds were removed by hand. No fertilizer or pesticides were applied, and plants were irrigated only during the first week after transplanting.

GROWTH TRAITS

At the end of the growing season, we counted tillers per plant in the field and harvested all aboveground biomass in October 2008 after a hard frost. We dried biomass for 3 days with forced air, separated panicles, and weighed both panicles and aboveground vegetative portions.

FOLIAR TISSUE CHEMISTRY AND DIGESTIBILITY

To assess traits associated with biomass recalcitrance and conversion efficiency, we compared tissue chemistry and digestibility among switchgrass populations sampled from the common garden experiment. For large herbivores and mechanical harvests that consume a mixture of stems and foliage, these parameters are determined both by leaf-to-stem biomass ratios as well as by tissue constituents (Twidwell et al. 1988). However, because virus-transmitting aphids are small, they can sample only one tissue type at a time. We therefore focused our analysis on foliar tissue because in our experience cereal aphids prefer leaves over stems in experimental situations (data not shown).

To evaluate tissue chemistry, we measured two aspects of cell wall components from leaf blade tissue: (1) acid detergent lignin (ADL) and (2) neutral detergent fiber (NDF). To determine lignin concentration, we followed the methods of Van Soest (1973). In brief, ground tissue samples were heated in acid-detergent (containing cetyl trimethylammonium bromide and sulfuric acid) and treated with 72% sulfuric acid. Silica content was accounted for by ashing at 550°C for 6 h. To determine NDF, we followed the methods of Mertens *et al.* (2002). Samples were refluxed in neutral-detergent solution (containing sodium hydroxide, EDTA, dibasic sodium phosphate, sodium borate decahydrate, and sodium lauryl sulfate) and α-amylase solution, and afterwards, remaining residues were washed with boiling water and then acetone. In biofuel feedstock development, reduction of lignin is predicted to increase conversion efficiency because lignin can block enzymatic digestion by encasing cell wall polysaccharides (Gomez et al. 2008); similarly, in forage crop development, reduced lignin can improve forage quality (Li et al. 2008). NDF is a broader measure of total insoluble fiber that includes cellulose, hemicellulose, as well as lignin. The cellulose and hemicellulose components of NDF provide the substrate for enzymatic hydrolysis, which then provides sugars for fermentation in biofuel production (Gomez et al. 2008).

As direct measures of biomass recalcitrance, we quantified both *in vitro* neutral detergent fiber digestibility (IVNDFD) and *in vitro* true dry matter digestibility (IVTDMD) of leaf tissue following the methods of Goering & Van Soest (1970). In brief, ground foliar tissue (0.5 g dry weight) was placed in a flask with a rumen buffer and mineral solution (containing rumen fluid collected from a rumen-fistulated dairy cow) and allowed to incubate in a shaking water bath at 40°C under carbon dioxide. The sample was then rinsed with neutral-detergent, treated with decahydronapthalene, and washed with boiling water and then acetone. *In vitro* TDM digestibility was calculated as 100 – percent dry residue; *in vitro* NDF digestibility, as percent NDF – percent dry residue. *In vitro* NDF digestibility measures how easily NDF is hydrolyzed by ruminal microbes over the expected retention time in the rumen (here, 48 h). High *in vitro*

NDF digestibility is associated with low lignification, improved forage quality, and greater cellulosic ethanol production (Allen 2000, Lorenz et al. 2009, Bals et al. 2010). *In vitro* TDM digestibility assesses to what extent total dry matter (which includes both cell contents and NDF) can be broken down by ruminal microbes.

Tissue chemistry and digestibility measures were conducted on leaf blade tissue from common garden plants harvested in 2008 (*n* = 9–10 per population). However, limits in foliar tissue from small plants reduced the number of individuals tested for lignin concentration in the MWF population (*n* = 7).

SUSCEPTIBILITY TO *BARLEY AND CEREAL YELLOW DWARF VIRUSUES*

Barley and cereal yellow dwarf viruses (*Luteoviridae*: BYDVs and CYDVs; hereafter B/CYDVs) are a group of aphid-transmitted +ssRNA viruses that infect wild and cultivated Poaceae species worldwide (Lister and Ranieri 1995). B/CYDVs are sometimes called the “yellow plague of cereals” (Conti et al. 1990) because of stunting and yield loss they cause in cereal crops (Jensen and D'Arcy 1995, McKirdy and Jones 2002), and their influence on wild grasses is of increasing interest. These viruses are emerging as key model systems in plant virus ecology (Power 1991, Malmstrom et al. 2006, Borer et al. 2007). Several virus species have been recognized, including BYDV-PAV and CYDV-RPV (Lister and Ranieri 1995).

The few previous studies investigating switchgrass susceptibility to B/CYDVs reported conflicting results. An early study did not find switchgrass to be susceptible to a B/CYDV vectored by *Rhopalosiphum padi* L. (bird cherry-oat aphid) (Stoner 1976), but more recent work using enzyme-linked immunosorbent assays (ELISA) found switchgrass plants infected with BYDV-MAV and BYDV-SGV in the tallgrass prairie in Kansas, U.S.A. (Garrett et al. 2004).

Like other perennial grasses, switchgrass has the potential to serve as a long-term reservoir for B/CYDVs and as a host for aphid vectors.

To better understand switchgrass susceptibility to B/CYDVs, we conducted three studies. First, to evaluate the extent to which switchgrass in Michigan develops B/CYDV infection under contemporary virus pressure, we used molecular diagnostics to quantify B/CYDV infection in six established switchgrass fields in Southern Michigan across a 300-km transect (Table 3.2). We harvested foliar tissue in September and early October 2008 from 30 switchgrass individuals along two 70 m transects through each field. We stored tissue samples at -20ºC until processing. Total RNA was extracted from 75 mg subsamples of tissue using Tri-Reagent (Sigma-Aldrich, Inc., St. Louis, MO, USA) and chloroform according to the manufacturer's instructions. To identify viruses, we used 1 μg of RNA in multiplexed reverse transcription polymerase chain reaction (RT-PCR) with primers that detect a wide range of B/CYDVs (following protocols (Malmstrom and Shu 2004). As per this protocol, we used SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) for RT and AmpliTaq Gold DNA Polymerase (Applied Biosystems, Carlsbad, CA, USA) for PCR.

Second, we evaluated infection rates from natural virus pressure in our common garden experiment. We sampled fully expanded leaves near the tops of tillers from individuals (*n* = 9–10 per population; 48 total samples) in the common garden experiment to compare field infection rates among varieties. Because virus analysis is best conducted on samples from physiologically active tissue, we sampled plants while they were still green in September 2008, after about three months of field exposure to natural aphid and virus populations. Samples were processed as above.

Following the discovery of B/CYDV infection in the field study, we decided to further quantify virus interactions by assessing rates of virus acquisition under uniformly high virus pressure. To do this, we caged viruliferous *R. padi* on individual greenhouse-grown switchgrass plants for six days and measured subsequent development of infection. *Rhopalosiphum padi* is the most common B/CYDV vector in our region (D. Voegtlin, North Central Regional Suction Trap Network, unpublished results) and can acquire and transmit multiple B/CYDV species (Irwin and Thresh 1990). We tested infection rates in the same switchgrass populations used in the common garden experiment, except for Southlow, which was dropped due to low germination rates.

For the inoculation tests, we cold-stratified switchgrass seeds for 2 weeks, planted multiple seeds into 12.7 cm plugs, and thinned seedlings to one individual per plug approximately 1.5 weeks later. Prior to inoculation, non-viruliferous *R. padi* were allowed to acquire BYDV-PAV from the local Great Lakes region by feeding for 24 hrs on infected *Avena sativa* leaves (Gray et al. 1991) from plants that had tested positive for infection using the RT-PCR assay. Next, five viruliferous aphids were caged on each switchgrass seedling (*n* = 28–38 per population) within 10.5 cm tall transparent cages, which covered each plant completely. Aphids had access to seedlings for six days (a long inoculation access period (Power et al. 1991)) before being killed with Astro insecticide (FMC Corporation, Philadelphia, PA, USA). Ten days later, we harvested the three youngest, fully-developed leaves from each plant. Plants from all populations were at similar developmental stages for inoculation and when tissue was harvested. Tissues were stored and processed as above.

APHID HOST PREFERENCES

To assess whether aphid host preferences explained differences in virus acquisition rates among switchgrass populations, we conducted a series of host choice tests in arenas in the laboratory. In these tests, 15 or 20 adult apterae (wingless aphids) were placed in a petri dish containing up to four equal-size pieces of tissue from leaves of different host populations and left in the dark for 24 hrs at 22ºC, after which the number of aphids on each leaf type was counted (following methods in (Malmstrom et al. 2005b). Tissue samples were taken from fully expanded, physiologically active leaves from the upper half of the larger tillers of each plant and were placed on moist filter paper in the petri dish in randomized order. Aphids were placed on the filter paper, not on leaves.

For context, we first compared *R. padi*'s relative preference between switchgrass and maize, using representative cultivars (switchgrass 'Dacotah' (Table 3.1) and maize hybrid 36R19). We used 4 cm x 0.5 cm leaf portions from 4-week-old maize and 5-week-old switchgrass, due to the slower nature of switchgrass growth. There were 15 replicate arenas containing 15 *R. padi* each.

Next, we compared *R. padi* feeding preferences among the field-grown switchgrass populations from the common garden experiment. In June 2009, we used 0.5 x 2 cm samples of green leaves from field-grown individuals from each of the four populations tested in the inoculation study. Aphid preferences were tested using 20 apterae per arena. The experiment was structured as a complete block design with 40 replicates.

Third, we used an additional arena study with 20 replicates to compare the attractiveness of 'Trailblazer' to that of three other switchgrass types commonly planted in our region: 'Cave-in-Rock', 'Pathfinder', and 'Shawnee' (Table 3.1). We obtained field-grown tissue for this study from a common garden established in 2006 by Suleiman Bughrara at Michigan State University's Crop and Soil Teaching and Research Center (East Lansing, MI). Tissue was collected from the second leaf from the top of tillers.

ANALYSIS

We compared growth parameters, tissue chemistry, and measures of biomass recalcitrance among switchgrass populations with ANOVA in Statistix 9.0 (Analytical Software, Tallahassee, FL, USA). Lignin concentration and IVNDFD were log transformed to meet assumptions of normality for all analyses. To reduce redundancy among switchgrass traits, we used principal components analysis (PCA) (R 2.11.1, R Foundation for Statistical Computing, Vienna, Austria). PCA is useful for assessing multiple plant traits (Oyarzabal et al. 2008), and we used it to compress seven trait variables: vegetative aboveground biomass, panicle biomass, number of tillers, NDF, IVNDFD, IVTDMD, and lignin concentration. As noted, some natural infection occurred in the common garden experiment during the course of the experiment. However, virus-infected plants did not differ significantly from uninfected plants for any of the traits measured (ANOVA, $P > 0.05$), so analyses reported include data for all plants.

To compare infection rates among populations, we used Fisher's exact test for data from the common garden study and a Chi-Square test in Statistix 9.0 for greenhouse inoculation data. We used ANOVA, also in Statistix 9.0, to evaluate aphid host preferences in arena tests.

Given the population differences that became evident in the trait analyses, we then explored potential relationships between biofuel-valuable traits and susceptibility. To assess which individual traits or which trait-space (based on principal components) may be associated with susceptibility to infection and attractiveness to aphid vectors, we used linear regression in R

2.11.1. Because virus and aphid interactions were assessed at the population level, we used population averages of trait values for regression. We then ranked traits based on their R^2 value, excluding traits that explained less than 10% of variation in the data.

Results

EFFECTS OF SELECTION ON SWITCHGRASS TRAITS

Contrary to our expectations, foliar digestibility and growth rates of switchgrass populations were not strongly associated and largely represented different trait axes. For example, principal component analysis of all measured traits found foliar digestibility measures to be the primary loading factors for PC1 (loading values: IVTDMD = 0.55; IVNDFD = 0.52; lignin = -0.41) whereas growth traits were the primary loading factors for the orthogonal PC2 (loading values: vegetative biomass = 0.52; panicle biomass = 0.62) (Fig. 3.1). With respect to the first principal component, the two near-wildtype populations (MWF, Southlow) grouped separately from the intensively selected cultivar, ‘Trailblazer’, while the two moderately selected varieties, ‘Blackwell’ and ‘Nebraska 28,’ occupied intermediate positions (Fig. 3.1).

Two-dimensional trait diagrams further highlight distinctions between digestibility and growth traits and reveal how switchgrass cultivars have been shaped along these axes by human selection. As expected, lignin concentration was a significant predictor of *in vitro* TDM digestibility (linear regression, R^2=0.22, P = 0.001), but lignin is relatively poorly predicted by aboveground biomass (linear regression, R^2=0.09, P = 0.045) (Fig. 3.2a&b). The trait spaces of the two near-wildtype populations (MWF, Southlow) were broad but centered in zones that represent less desirable values for biofuels (less biomass, lower digestibility, more lignin) (Fig. 3.2b&c). Selection pressure for increased yield and forage value were evident in the shift of the

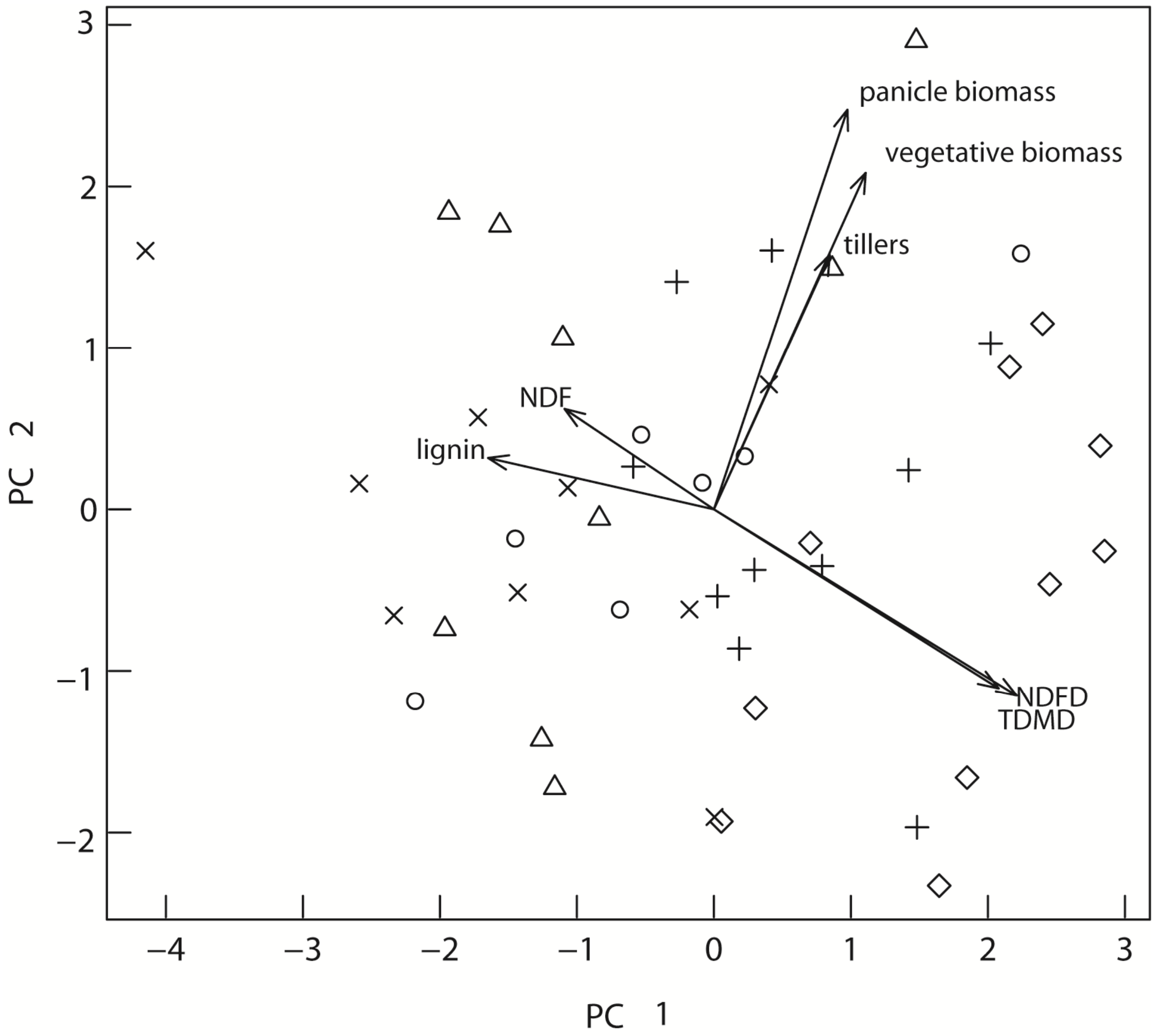

Fig. 3.1. Distribution of switchgrass individuals according to principal components 1 and 2. Each point marks an individual plant (○ = MWF, Δ = Southlow, + = ‘Nebraska 28, x = ‘Blackwell’, and ◊= ‘Trailblazer’). Arrows represent relative loadings of trait values on the principal component axes.

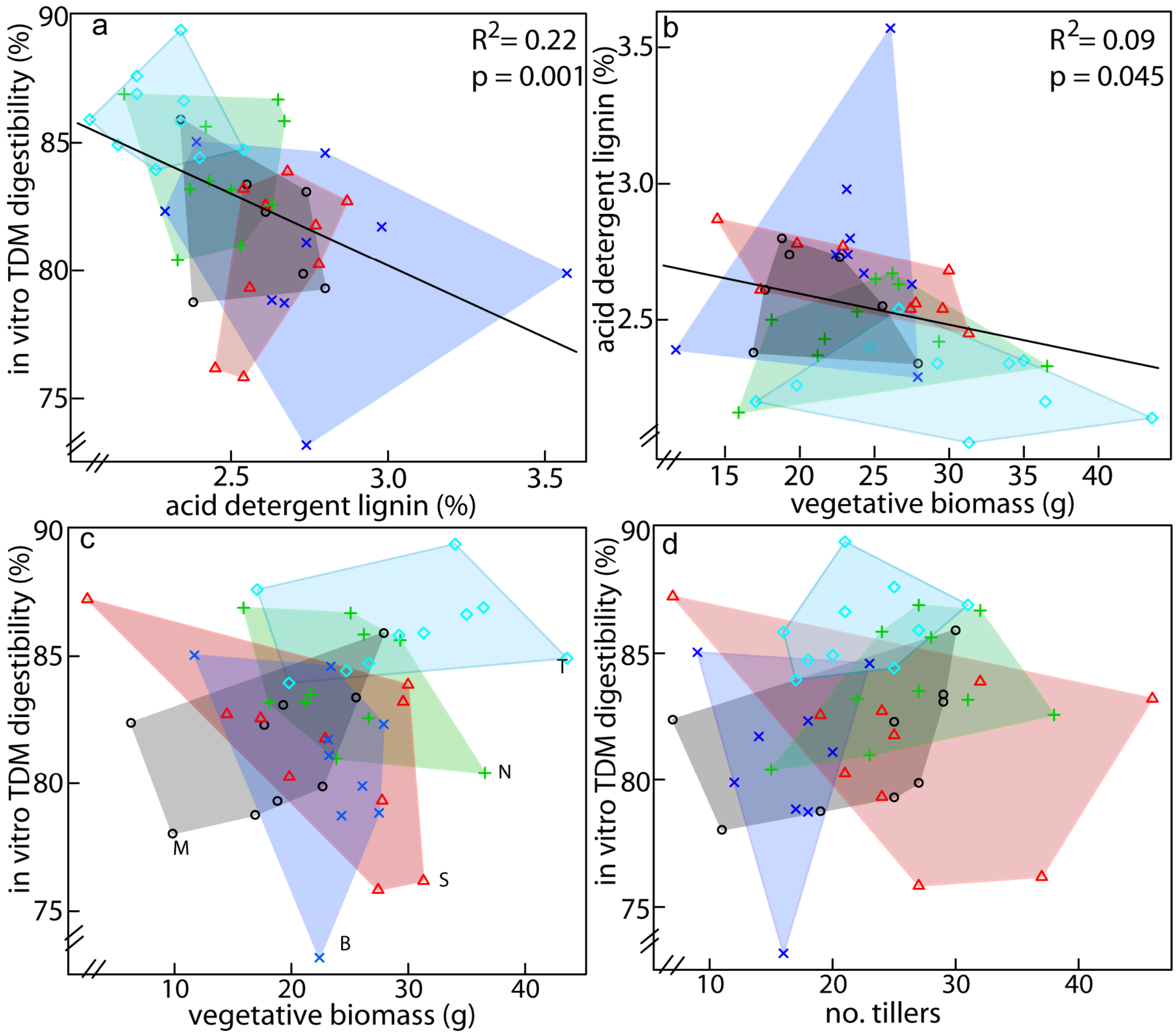

Fig. 3.2. Trait associations between (a) lignin and IVTDMD, (b) vegetative biomass and lignin, (c) vegetative biomass and IVTDMD, and (d) tiller number and IVTDMD. Each point designates an individual switchgrass plant (○ = MWF, Δ = Southlow, + = 'Nebraska 28, x = 'Blackwell', and ♢ = 'Trailblazer'). Trait space of each population is shown in shaded minimum convex polygons. Lignin data was unavailable for three individuals, resulting in fewer points in (a) and (b). For interpretation of the references to color in this and all other color figures, the reader is referred to the electronic version of this dissertation.

trait space of cultivar ‘Nebraska 28’ towards the upper right in comparison with the near-wildtype populations (Fig. 3.2c). This shift continues further with ‘Trailblazer’, a cultivar subject to intense selection for forage quality, and in which foliar digestibility was uniformly high. Biomass accumulation rates in 'Trailblazer' were also high but more variable, suggesting primacy of selection for tissue digestibility. In contrast, ‘Blackwell’ diverges from this selection trajectory. This cultivar appears to have been shaped for increased forage value along a different pathway: through increased ‘leafiness’ and reduction of tiller number. Thus, these two cultivars inhabit nearly orthogonal spaces in an *in vitro* TDM digestibility x tiller number comparison: 'Trailblazer' is most uniform in *in vitro* TDM digestibility, 'Blackwell' in tiller number (Fig. 3.2d). Both selection pathways lead to increased forage value, but by different means.

On a single trait basis, selection pressures have created continuums of trait differences from wildtype to highly selected, as evidenced by accumulation rates of aboveground vegetative biomass during the first growing season (ANOVA, $F_{4,43} = 3.25$, $P = 0.02$) (Fig. 3.3a). Populations also differed significantly in tiller number (ANOVA, $F_{4,43} = 4.1$, $P = 0.007$) (Fig. 3.3b), foliar lignin concentration (ANOVA, $F_{4,40} = 6.8$, $P = 0.0003$) (Fig. 3.3c), foliar NDF concentration (ANOVA, $F_{4,43} = 2.8$, $P = 0.04$) (Fig. 3.3d), *in vitro* NDF digestibility (ANOVA, $F_{4,43} = 2.8$, $P = 0.002$), and *in vitro* TDM digestibility (ANOVA, $F_{4,43} = 5.2$, $P = 0.0004$) (Fig. 3.3e&f). On average, ‘Trailblazer’ was more digestible than both near-wildtype populations and 'Blackwell', but not 'Nebraska 28' (Tukey HSD $P < 0.05$).

In tiller and digestibility measures, the differing nature of selection pathways used to improve forage quality is evident in the divergence of 'Blackwell's values from trends among the other populations (Fig. 3.3).

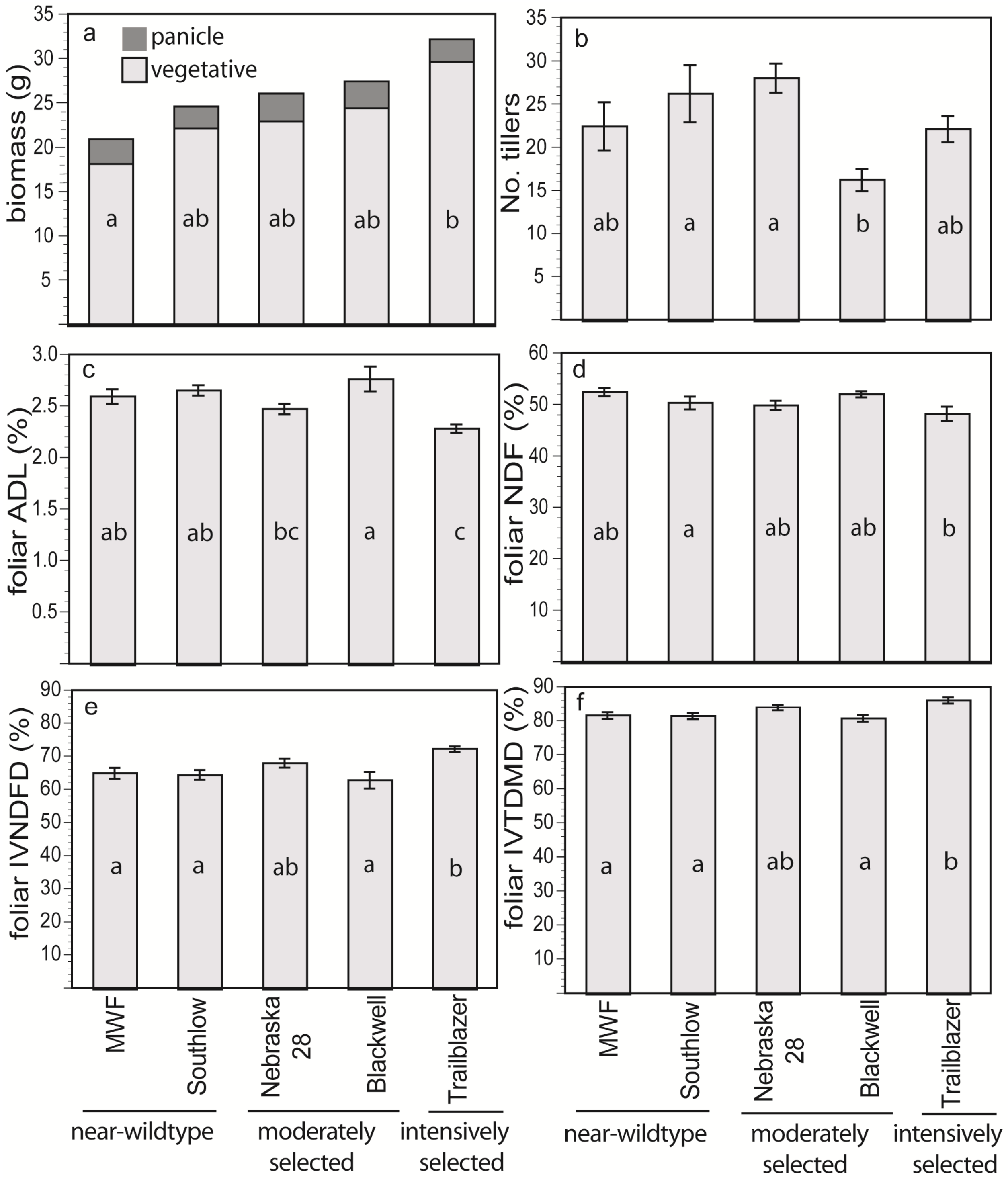

Fig. 3.3 Growth trait differences among populations of switchgrass. (a) Aboveground biomass, both vegetative mass (light bars) and reproductive panicles (dark bars). Significant differences are given for vegetative biomass only; panicle biomass differences were not significant. (b) Tiller number. (c) Foliar lignin concentration. (d) Foliar NDF concentration. (e) Leaf tissue digestibility as measured by IVNDFD and (f) IVTDMD. Different letters indicate significant differences at $\alpha = 0.05$. Error bars indicate one standard error.

SUSCEPTIBILITY TO VIRUS INFECTION

Our findings indicate that switchgrass can accumulate B/CYDV infection quickly under natural virus pressure. In fields (ca. 6–18 yrs old) across a transect in southern Michigan, B/CYDV prevalence ranged from 0–28% (Table 3.2). On a shorter timescale, the initially virus-free switchgrass individuals in our common garden accumulated 10.6% incidence of B/CYDV infection (5/47 plants sampled) in just three months of field exposure in 2008 (Table 3.3). Infected plants showed few signs of the B/CYDV-induced discoloration typically evident in cultivated cereals. Cultivars appeared to be more susceptible to virus infection than near-wild-type populations. In our common garden experiment, none of the near-wildtype plants were infected in the first year, whereas five cultivar individuals were (Fisher's exact test, $P = 0.07$). Of these, two were infected with BYDV-PAVs and three with CYDV-RPVs.

In the greenhouse trial, infection rates were higher overall, averaging 38% incidence across all populations. As in the field trials, near-wildtype plants (MWF) experienced the lowest infection rates (12%), and were less likely to be infected than cultivars ($\chi^2 = 10.9$, $df = 1$, $P = 0.0009$) (Table 3.3). At the other extreme, BYDV incidence in the fast-growing, highly digestible forage cultivar 'Trailblazer' was 68%—5.5 times greater than in MWF ($P < 0.05$) and 2.1 times greater than in 'Nebraska 28' (32% infection incidence) ($P < 0.05$).

APHID HOST PREFERENCES

Rhopalosiphum padi, the primary vector of several B/CYDVs in our region, will feed on switchgrass but strongly prefers maize when given a choice of foliar tissue. For example, in our arena tests, approximately 3.5 times more *R. padi* preferred maize tissue (hybrid 36R19) over switchgrass ('Dacotah'; ANOVA, $F_{1,28} = 51.3$, $P < 0.0001$).

Table 3.2. B/CYDV prevalence in established switchgrass fields in Southern Michigan in 2008 as quantified with RT-PCR.

				B/CYDV prevalence	
County	Cultivar	Stand age (yrs)	Field size (ha)	(n/n)	(%)
Allegan	Unknown	8	2.4	0/30	0
Tuscola	Unknown	6	14.2	0/27	0
Cass	Forestburg	8	2.8	3/30	10
Saginaw	Unknown	8-18	4	3/30	10
Barry	Cave-in-Rock	Unknown	6.1	8/30	27
Kalamazoo	Cave-in-Rock	7	3.2	8/29	28

Table 3.3. B/CYDV prevalence in switchgrass populations (1) in the field under natural virus pressure and (2) in the greenhouse inoculation, as quantified by RT-PCR.

		B/CYDV Prevalence			
		Field Experiment		Greenhouse Inoculation	
Population	Type	(n/n)	(%)	(n/n)	(%)
MWF	Near-wildtype	0/9	0	4/33	12^{a}
Southlow	Near-wildtype	0/10	0	-	-
Nebraska 28	Cultivar	3/8	37.5	12/38	32^{a}
Blackwell	Cultivar	1/10	10	11/29	$38^{a,b}$
Trailblazer	Cultivar	1/10	10	25/37	68^{b}

Different letters indicate significant differences for percent infection in the greenhouse inoculation.

In arena comparisons among switchgrass populations examined here, *R. padi* strongly preferred leaves from two cultivars noted for good forage production (‘Trailblazer’ and ‘Blackwell’; Table 3.1) over leaves from ‘Nebraska 28’ or the near-wildtype population (MWF) (Tukey HSD $P < 0.05$) (Fig. 3.4a). In comparisons with other widely planted switchgrass cultivars, 'Trailblazer' was comparable in preference to 'Shawnee', ‘Pathfinder,’ and 'Cave-in-Rock'. Interestingly, 'Shawnee', which was developed for increased tissue digestibility from ‘Cave-in-Rock’ (Vogel et al. 1996), attracted 3 times more aphids than ‘Cave-in-Rock’ (Tukey HSD, $P < 0.05$). Since our field surveys detected notable B/CYDV prevalence in 'Cave-In-Rock' under natural virus pressure (Table 3.2), this aphid preference suggests that 'Shawnee' might suffer similar or greater pressure in the field.

PREDICTORS OF VIRUS AND APHID SUSCEPTIBILITY IN SWITCHGRASS

Among the plant traits measured, mean vegetative biomass accumulation per individual in the field was the best predictor of population-level susceptibility to virus in the greenhouse inoculation (linear regression, $R^2 = 0.99$, $P = 0.006$) (Fig. 3.5; Table 3.4). While other traits may also influence susceptibility, none were significant within the limits of detection. Lignin concentration, for example, was not significantly related to population-level susceptibility (linear regression, $R^2 = 0.46$, $P = 0.32$) (Fig. 3.5).

When traits were ranked according to the amount of variability they explained, rate of vegetative biomass accumulation, followed by measures of tissue digestibility and cell wall traits, explained the most variability in BYDV-PAV susceptibility (Table 3.4). Differences in tillering strategies explained the most variability among switchgrass populations in attractiveness to aphids, with *R. padi* tending to prefer populations that produced fewer tillers per individual.

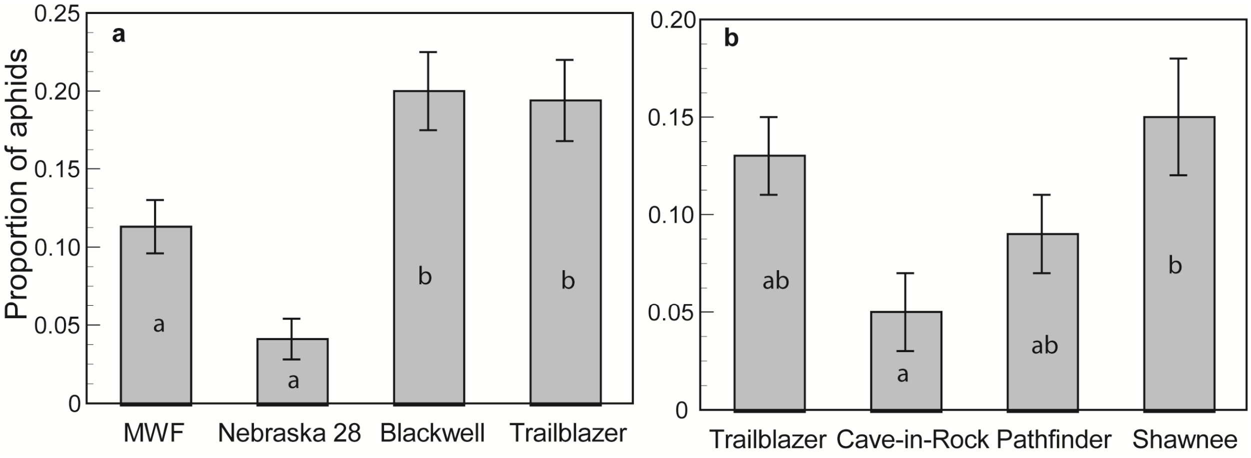

Fig. 3.4. Aphid host preferences (a) among leaf tissues of field-grown switchgrass populations from our common garden experiment and (b) among field-grown tissue of four common cultivars of switchgrass. Different letters indicate significant differences at α = 0.05. Error bars indicate one standard error.

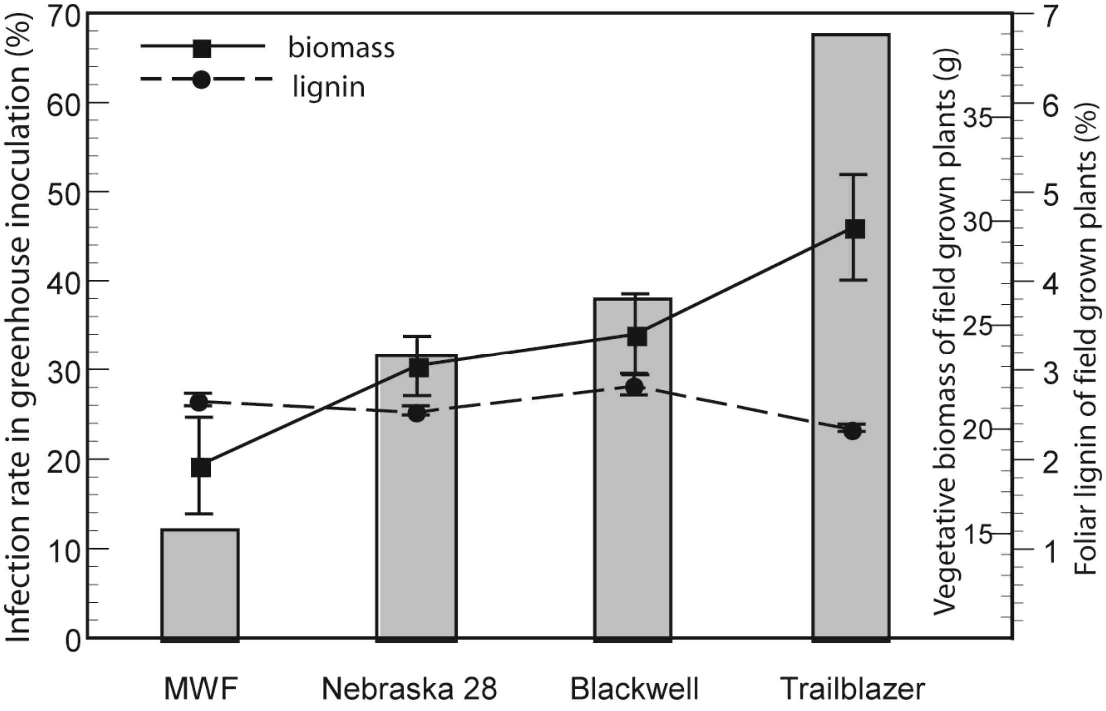

Fig. 3.5. Relationship between switchgrass virus susceptibility in greenhouse inoculation trials and biofuel-valuable traits as measured in the common garden (see results for R^2 values). Biomass and lignin values are population averages, and error bars indicate one standard error.

Table 3.4. Predictors of population-level virus susceptibility and aphid preferences, ranked by R^2 values in linear regression. Traits are described from field-grown populations.

	Virus susceptibility	Aphid preference
Vegetative biomass accumulation	1*	3
IVNDFD	2	-
IVTDMD	3	-
Lignin	4	-
NDF	5	-
Panicle biomass	6	2
No. tillers	-	1

* Significant regression

-, Traits with $R^2 < 0.1$ are not ranked

Digestibility and cell wall traits had essentially no influence on aphid preferences (Table 3.4). Principal component values, although they incorporated multiple traits, were not significantly associated with virus and aphid interactions (linear regression, $P > 0.05$).

Discussion

Viruses likely have infected crops since the beginning of agriculture (Stukenbrock and McDonald 2008, Gibbs et al. 2010). In many locations and periods, humans have sparked serious crop epidemics through intensification of agriculture (Thresh 1982), transport of crops into new regions (Thresh 1980, Jones 2009), and specific modifications of plant traits (Thresh 1982). Our findings here suggest that, without mitigation, deployment of new bioenergy crops could perturb virus epidemiology and possibly increase risk of regional virus spread. Of particular concern is the indication that selection for biofuel-valuable traits could increase disease susceptibility in perennial species that already have the potential to serve as long-term pathogen reservoirs. Our study suggests a framework for risk assessment of pathogen interactions with novel biofuel crops; future population-level studies will expand understanding of these interactions and provide a basis for mitigation strategies.

In the last century, humans began a renewed campaign of selecting native species for use in working landscapes and for ecological restoration (Vogel 2000, Sanderson et al. 2004)). The effort to domesticate wild plants is now intensifying to meet human demand for renewable energy sources (Casler 2010). As in past domestication events (Vasey 1992, Ladizinsky 1998), a substantial effort is focused on increasing plant growth rates and improving the quality of tissue for consumption. Historically, the consumers for which new crops were selected were humans and livestock, but changes in crop traits that benefited these target consumers often inadvertently benefited other consumers, including pathogens and insect pests (Thresh 1982). At present, the target consumers for cellulosic biofuels are combustion engines and the upstream feedstock processing units needed to recreate or replace the digestion of cellulosic material by ruminal microbes. Like ruminants, these industrial consumers will benefit from highly productive, easily digestible feedstocks (Lorenz et al. 2009). Thus, selection of new biofuel crops retraces or extends in many aspects the trajectory of past domestication events and may exert analogous influences on pathogen communities.

To date, work on pathogens in biofuel crops has focused on disease identification (Crouch et al. 2009, Agindotan et al. 2010, Gao et al. 2010). Our findings demonstrate the need to quantify how different selection pressures aimed at reduced biomass recalcitrance or increased productivity will influence a broad range of pathogen types, specifically including viruses and the phloem-feeding insects that transmit many of them.

In selection of biofuel crops, the aim of reducing lignin and other constituents that contribute to biomass recalcitrance has spurred conversations about potential consequences for stem strength and plant hardiness (Casler et al. 2002, Pedersen et al. 2005). High lignin levels are associated with decreased forage intake (Forbes and Garrigus 1950) and decreased attractiveness to grazers (Cherney et al. 1990, Aregheore et al. 2006). Thus, reducing lignin might increase plant vulnerability to mammalian and other grazers. In switchgrass, however, we found that growth rates better predicted aphid preferences and virus susceptibility than did lignin levels. Similarly, foliar lignin levels did not explain plant resistance to another phloem-feeder, *Blissus insularis* (Hemiptera: southern chinch bug), in St. Augustinegrass (Rangasamy et al. 2009).

The linkage we found in switchgrass between fast growth rates, virus susceptibility, and aphid attractiveness may be only partly explained by an understanding of trade-offs between growth and defense in plant allocation. In general, fast growth is associated with short life-cycles and reduced investment in defense, such as lignin and polyphenols, whereas slow growth is associated with longevity and greater investment in defenses (Coley et al. 1985). In *Arabidopsis thaliana*, for example, individuals with natural constitutive expression of a defense pathway were slower growing than plants lacking this allele (Todesco et al. 2010). In grasses, fast growth is one hallmark of a "quick return" species that may disproportionately serve as effective pathogen hosts (Cronin et al. 2010). However, the trait analysis presented here demonstrates that, in switchgrass, growth rates and foliar digestibility measures (including lignin concentration) are not strongly linked and, in fact, represent dominant components on two separate axes that are orthogonal to each other. Selection pressures have acted on both these axes to varying degrees in different cultivars. A key priority for future research, therefore, is to elucidate mechanisms that underlie the linkages between growth rates and different types of plant defense, both physical and chemical.

That growth rates per se appear to influence virus and aphid susceptibility may be best understood in the context of how these organisms interact with hosts. Whereas lignification strongly influences the quality of tissue that grazers must chew and digest, phloem-feeding insects, such as aphids, must only puncture cell walls to gain access to phloem (Tjallingii and Hogen Esch 1993) and then digest liquid phloem contents. As a result, fast host growth rates may be important promoters of fitness in phloem-sucking insects (Grechi et al. 2008, Sauge et al. 2010) that can benefit from increased rates of nutrient translocation (White 1993). In parallel, increased host growth rates may benefit viruses by providing more opportunities for replication and expression as a function of their dependence on the host's cellular machinery (Whitham and Wang 2004). Plant resistance to B/CYDV infection may be a function of interactions with aphid vectors, through effects on virus transmission, and with viruses, through interference with viral replication (Qualset et al. 1990).

RISK ASSESSMENT AND IMPORTANCE OF LANDSCAPE CONTEXT

In our study, the majority of the switchgrass fields surveyed showed some level of B/CYDV infection, suggesting that these fields have potential to serve as virus reservoirs. This potential is underscored by recent discoveries of novel marafi-like viruses (Tymoviridae) in similar fields (Agindotan et al. 2010, A. Schrotenboer & C. Malmstrom, unpublished results). The effects of these viruses on biofuel crop yield and their potential for spillover into other regional crops are therefore of great interest.

B/CYDVs have been shown to significantly reduce biomass production in California native perennial grasses (Malmstrom et al. 2005a) and in *Miscanthus sinensis*, an Asian perennial grass under consideration as a biofuel feedstock (Huggett et al. 1999), and marafiviruses can cause substantial yield loss in maize (Gámez 1976, 1983). However, the influence of these species on tallgrass prairie species is poorly understood. Based on current knowledge, it is reasonable to predict that consequences of virus interactions with native prairie grasses may decrease productivity as well. Consistent with this prediction, insecticide application to switchgrass increased yields by 11% (C. Gratton, unpublished results). Nonetheless, it is important to consider the alternative that virus influence on native grasses might be neutral to positive. Although little is understood about plant-virus interactions in nature, a few studies indicate that some viruses promote fitness in stressful environments (Gibbs 1980, Márquez et al. 2007, Xu et al. 2008).

The larger concern is the potential for spillover from biofuel grasses to food crops and other vegetation types that are virus-susceptible. Because B/CYDVs and many other plant viruses are transmitted by sucking insects that can travel long distances (Irwin and Thresh 1990), the development of biofuel grasses as virus reservoirs could change pathogen dynamics in a broad area. The likelihood of this occurring will be determined by a series of interacting factors, including vector transmission efficiency and dispersal patterns and the counteracting influence of biocontrol services in perennial stands (Gardiner et al. 2010, Landis and Werling 2010), which merit further investigation.

Here, our finding of linkage between growth rates and virus susceptibility in switchgrass highlights the potential for biofuel cultivar selection to inadvertently increase virus reservoir capacity. Disease amplification by fast-growing plant species has been documented in other ecosystems. For example, fast-growing, susceptible annual hosts can increase B/CYDV incidence in other, less susceptible species (Power and Mitchell 2004, Malmstrom et al. 2005a, Malmstrom et al. 2005b). Proactive steps to prevent or mitigate pathogen susceptibility prior to widespread use of newly developed biofuel crops could aid in averting potential unintended consequences of biofuel production on pathogen dynamics.

CHAPTER 4

LANDSCAPE AND COMMUNITY INFLUENCES ON VIRUS VECTORS IN BIOFUEL CROPS

Abstract

Numerous historical examples demonstrate that changes in agricultural practices and land cover patterns can alter pathogen dynamics. Current expansion of bioenergy production is driving widespread development of novel cropping systems and land cover change, thus producing conditions that could alter pathogen pressure. Of particular concern is the potential for changes in the dynamics of plant viruses that could be moved among vegetation types by highly mobile vectors responsible for dispersing many plant viruses. Deployment of perennial biofuel crops has potential either to increase or decrease virus and vector pressure, depending upon interacting effects of community and landscape dynamics. A key unknown is the influence of biodiversity—both at the scale of the community and of the surrounding landscape—on vector and virus pressure. Here, we examine the effects of community composition, landscape patterns, and environmental factors on virus prevalence and vector pressure in three biofuel crops—maize, switchgrass (*Panicum virgatum*), and mixed prairie—in the US Great Lakes region. In sites along a 300-km transect in Michigan, we quantified the prevalence of aphid-vectored *Barley and cereal yellow dwarf viruses* in switchgrass, and we measured aphid pressure using bowl traps, sticky card traps, and suction traps. We quantified plant community richness, landscape diversity, and proportions of land cover types at different scales and then used model selection to determine which factors best accounted for patterns of vector pressure. We found that landscape diversity, local species richness, and temperature were the most important determinants, with the effects varying by season. For example, spring vector pressure experienced by biofuel crops

increased as landscape diversity fell, but late summer vector counts showed the opposite relationship. To be most effective, management of viruses in agricultural landscapes requires mechanistic understanding of the influence of biodiversity at both landscape and local scales on vector pressures.

Introduction

Expansion of the bioenergy industry is driving changes in agricultural practices and land cover worldwide (Searchinger et al. 2008, Fischer et al. 2010). In the United States, for example, the federal government envisions replacing 30% of petroleum consumption with biofuels by 2030, which is estimated to require use of one billion dry tons of biomass feedstock per year (Perlack et al. 2005). To meet this goal will require reallocation of lands to biofuel crop production (Heaton et al. 2008, Conner and Hernandez 2009) as well as use of additional biomass sources, such as crop residues from food agriculture (Perlack et al. 2005).

In the past, other similarly wide-scale changes in agricultural practices and land cover have fostered virus emergence and disease outbreaks (Thresh 1982, Anderson et al. 2004, Jones 2009). For example, land use changes during the dawn of agriculture likely helped drive the emergence and radiation of pathogenic plant viruses (Gibbs et al. 2010), including potyviruses that today cause significant crop damage (Gibbs et al. 2008). This early expansion of viruses may have been related to greater availability of hosts for insect vectors (Gibbs et al. 2010). Because vectors disseminate many plant viruses through both short- and long-distance movements (Kring 1972, Irwin and Thresh 1990), factors influencing their movements and abundance can alter virus prevalence and severity (Burdon and Chilvers 1982, Power 1991). As a result, changes in agricultural practices including increased use of monocultures and fertilization, can increase both vector abundance and the extent of virus outbreaks (Seal et al. 2006). For these reasons, development of sustainable biofuel landscapes requires consideration of the potential impact of changes in cropping strategies on virus dynamics and methods for mitigating any possible negative consequences.

In this study we focus on aphids that vector a group of generalist plant viruses, *Barley and cereal yellow dwarf viruses* (*Luteoviridae*: BYDVs and CYDVs, hereafter B/CYDVs), in Poaceae biofuel crops in the Midwestern United States. We assess how community composition and landscape characteristics influence the likelihood of vector interactions with biofuel crops. B/CYDV infection causes major yield losses in cereal crops worldwide (Lister and Ranieri 1995, McKirdy and Jones 2002), and also can occur in many wild species (Griesbach et al. 1990, Garrett et al. 2004, Malmstrom et al. 2005a). Thus, B/CYDV interactions with bioenergy crops offer contemporary opportunities to assess relationships among plants, highly mobile insect vectors, and pathogens. To assess both current and future biofuel crops, we investigated aphid and virus interactions with maize and with two perennial systems—switchgrass (*Panicum virgatum* L.) and mixed prairie.

Because most studies of insect pest regulation have focused on interactions with annual food crops (Bianchi et al. 2006), little is known about interactions with wild perennial species. Maize-derived grain ethanol is currently the most common source of liquid biofuel in the United States (de Vries et al. 2010), but perennial systems are expected to be an integral part of second-generation biofuels made from cellulosic biomass (Gomez et al. 2008, James et al. 2010). In addition to serving as effective biofuel feedstocks, these perennial crops can provide ecosystem services, such as carbon sequestration, and reduce the need for agricultural inputs in comparison with current grain-based biofuel crops (Robertson et al. 2008). However, perennial grasses may share generalist pests and pathogens with cereal crops (Huggett et al. 1999, Spencer and Raghu 2009, Schrotenboer et al. 2011), which may allow pathogen exchange among biofuel and food crops.

A key issue is how community composition, landscape patterns, and environmental factors interact to determine vector and virus pressure in landscapes with perennial grasses. In the North American Midwest, a temperate-climate region historically dominated by perennial grasses and seasonal forest and now the center of U.S. maize and soy production, B/CYDV epidemiology has not previously been examined in a landscape context. In temperate Europe, another region in which perennial grasses may act as virus reservoirs, research has identified a wide range of environmental factors and landscape properties that shape B/CYDV prevalence, including climate (particularly temperature), topography, distance to roads, and surrounding land use (Foster et al. 2004, Jones et al. 2010). In contrast, in water-limited Mediterranean annual grasslands in southwest Australia, rainfall was found to be among the most important predictors of vector abundance and B/CYDV outbreaks (Thackray et al. 2009, Jones et al. 2010). In Mediterranean annual grasslands in the western U.S., local community composition was found to be more influential than regional-scale factors in determining B/CYDV infection rates (Borer et al. 2010), but influences on vector dynamics were not investigated.

In temperate regions, recent entomological studies have found landscape properties to be highly important drivers of the abundance of pests and natural enemies (Roschewitz et al. 2005, Gardiner et al. 2009a), and likely influence B/CYDV vectors as well. A critical component of this work is a focus on the influence of landscape diversity or complexity on insect activity. For example, in a meta-analysis of the effects of landscape complexity, more than 70% of the studies indicated that natural enemies were more abundant in landscapes with greater complexity

(Bianchi et al. 2006). The consequences for pest insects (including vectors) are less clear—only 45% of studies found that pest pressure was reduced in more complex landscapes (Bianchi et al. 2006).

Development of biofuel landscapes has potential both to increase or decrease diversity at community and landscape scales, depending upon policy and management decisions. For example, biofuel crops could homogenize landscapes if a single major biofuel crop is favored (Landis et al. 2008). Alternatively, biofuel strategies that incorporate polycultures could increase diversity both within communities and across landscapes. For example, mixes of tallgrass prairie species have been suggested as candidate feedstocks on marginal land (Tilman et al. 2006), and other forms of polyculture, such as mixed grass stands or intercropping, may be implemented as well (Hallam et al. 2001, Mulkey et al. 2008). At the landscape scale, use of several different feedstock crops could further increase diversity.

Within-field or local community diversity has been recognized for some time as a controlling factor for pests and pathogens. Within crop systems, polyculture can reduce abundance of insect herbivores (Risch et al. 1983), and genetic diversity within a single-species planting can reduce disease incidence (Power 1991, Mundt 2002). In more natural systems, increased species richness within plant communities has been associated with reduced disease severity by specialist fungal pathogens (Knops et al. 1999, Mitchell et al. 2002, Mitchell et al. 2003). In the latter studies, this effect was largely mediated by decreased density of host species, as suggested by Burdon and Chilvers (1982), but in some cases, an additional diversity effect independent of density was also identified (Mitchell et al. 2002, Mitchell et al. 2003).

The importance of landscape diversity in controlling pests and pathogens has been recognized only more recently. Recent work in the U.S. Midwest has demonstrated that greater landscape diversity is associated with increased biocontrol services (Gardiner et al. 2009a). Likeswise, numbers of soybean aphids (*Aphis glycines*) were found to be lower in soybean fields located within areas of high landscape diversity (Noma et al. 2010).

To investigate virus and vector pressure in Poaceae biofuel crops, our objectives in this study were to (1) determine the extent and geographic distribution of naturally-occurring B/CYDV infection in the biofuel crop switchgrass in our study region and (2) evaluate the relative influence of landscape properties, community composition, and environmental factors on vector pressure during key periods of vector dispersal. This study provides a novel investigation of virus and vector interactions with perennial grasses, and it advances understanding of the influence of diversity at community and landscape scales on plant virus ecology.

Methods

GENERAL APPROACH

Overall, our approach was to quantify virus and vector pressure within study fields and then evaluate potential factors influencing these interactions. We began by evaluating the extent to which one of the major perennial grasses, switchgrass, was infected with B/CYDVs, and we then assessed the mechanism for virus dispersal—alates (winged aphids). We consider our measures of alates moving in the landscape and in our study fields to be measures of vector pressure.

STUDY REGION

This study was conducted in the lower peninsula of Michigan in the Great Lakes region, where perennial grasses are expected to play a primary role in providing feedstocks for cellulosic biofuels (Dohleman et al. 2010). In 2008, thirty sites were established along a broad 300-km transect in southern lower Michigan (Fig. 4.1a) for studies of biofuel sustainability (Gardiner et al. 2010). In the 1800's, this region of Michigan included prairie, savanna, and woodland (Packard and Mutel 1997), which was subsequently converted to agriculture and urban uses in many areas as human population increased (Whitney 1994). The study sites included ten each of maize, switchgrass, and restored mixed prairie, and ranged in size from 2.5 to 32 ha. The prairie and switchgrass sites had been previously planted on former agricultural lands. The prairie sites, largely dominated by big bluestem (*Andropogon gerardii* Vitman), had been planted in 1999-2004, and the switchgrass fields had been planted in 1990-2002. In 2009, two switchgrass sites that had been sampled for virus in 2008 were removed from the study due to low switchgrass abundance and replaced with alternative switchgrass sites.

DOCUMENTING PRESENCE OF B/CYDVs

To determine the extent to which a major perennial biofuel species was infected with B/CYDVs, we sampled seven switchgrass fields, which had been established 6–18 years earlier, for virus incidence in 2008. We established two 70 m transects through each field and collected leaf tissue from individuals at 5 m intervals along each transect, for a total of 30 samples. Four upper leaves from one tiller of each sampled individual were harvested in September and frozen at -20°C for later processing. In some fields, low switchgrass density resulted in fewer than 30 samples.

To determine infection status of sampled plants, we followed the methods of Malmstrom & Shu (2004). We extracted total RNA from 75 mg of frozen samples using Tri-Reagent (Sigma-Aldrich, Inc., St. Louis, MO, USA) and chloroform and then identified viruses with

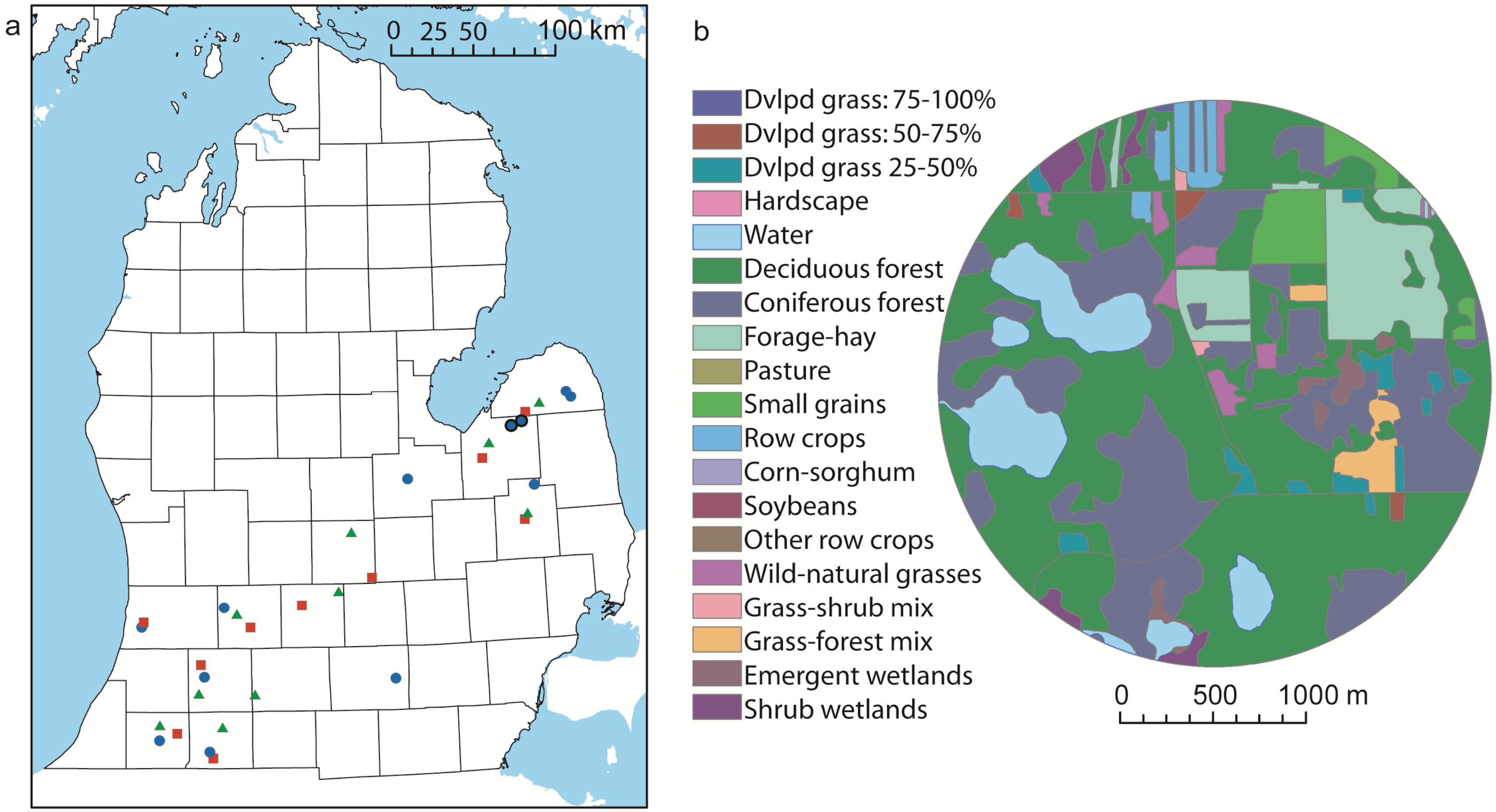

Fig. 4.1. (a) Locations of study sites in the lower peninsula of Michigan, USA. (Δ maize, ○ switchgrass, □ prairie) Switchgrass sites in dark outlines were sampled in 2008 only. (b) Representative example of land cover classifications around one study field using interpretation of NAIP imagery.

multiplexed reverse-transcription polymerase chain reaction (RT-PCR) that can detect and identify a broad range of B/CYDVs.

VECTOR MEASURES

To evaluate vector pressure, we used suction traps to capture alate aphids in the air stream at 6 m and two types of traps at lower levels in studied fields. During the spring migration period, alates fly long distances to find summer hosts (Vickerman and Wratten 1979). Thus, data from suction traps quantify synoptic aphid movements, whereas data from field traps quantify alate activity in areas where aphids are landing and interacting with potential host plants.

Suction traps

We analyzed aphid counts from the North Central Regional Soybean Aphid Suction Trap Network (www.ncipmc.org/traps/). These 6 m tall suction traps suck aphids from the air stream with a vacuum, from late spring to early fall in several Midwestern states. Aphids were identified to species (voucher specimens deposited at Illinois Natural History Survey's Insect Collection by D. Lagos). We used data from five suction traps in Michigan and two in northern Indiana to determine regional patterns of vector pressure. In this region, cereal aphid abundance generally shows two peaks—one in spring and one in late summer/early fall. We selected the dates with peak numbers of cereal aphids in spring of 2008 and 2009 to compare aphid abundance among traps. Cereal aphids included *Rhopalosiphum insertum* (Walker) (apple-grain aphid), *R. maidis* (Fitch) (corn leaf aphid), *R. padi* (L.) (bird cherry-oat aphid), *R.*

rufiabdominalis (Sasaki) (rice root aphid), *Schizaphis graminum* (Rondani) (greenbug), and *Sitobion avenae* (F.) (English grain aphid).

Field traps

In 2009, we deployed two forms of traps to catch winged aphids within field sites: yellow bowl traps and yellow sticky card traps. Bowl and sticky card traps capture different aspects of vector dynamics. In general, horizontal traps (like our bowl traps), mainly catch down-welling alates landing in a field, whereas vertical traps (like our sticky card traps) catch a greater proportion of alates moving within a field (Heathcote 1957).

Bowl traps filled with 25% propylene glycol were mounted on posts ~0.5 m above the vegetation canopy and were raised as canopies grew taller. Horizontal surface area for each bowl trap was ~145 cm^2. We established four traps in each field, at least 40 m from field edges and from each other. We removed insects and replaced propylene glycol weekly in the late spring from June 8-29 and in late summer from August 3-31. The last sampling period was extended to 2.5 weeks from August 31 – September 18, during which period 50% propylene glycol was used. Total aphids per site were averaged to estimate aphids per week. Using bowl traps, we sampled five maize, seven switchgrass, and six prairie sites in June, and we sampled all sites in the network (10 of each crop type) in late summer. Vertical sticky card traps (~400 cm^2 surface area; Great Lakes IPM, Vestaburg, MI) were mounted on posts at a height of 1 m above the ground, in four locations per field. We retrieved sticky card traps on 8 June 2009 after one week in the field from nine maize, ten switchgrass, and ten prairie sites.

All aphids in bowl and sticky card traps were counted. Aphids in sticky card traps were further separated into cereal aphids or other species. Because the results of analyses based on cereal aphids and on total aphids from sticky cards traps were similar, we therefore present values only for cereal aphids, unless otherwise noted.

INTERPOLATION OF VIRUS AND VECTOR DATA

To interpolate the spatial distribution of aphid and B/CYDV data, we used inverse distance weighting in ArcGIS 9.3 (ESRI, Redlands, CA, USA), which uses a weighted sum of known points to estimate values for unknown points (Chang 2004). To evaluate the extent of any spatial autocorrelation, we calculated Moran's I from observed data for aphid incidence in R 2.11.1 (R Foundation for Statistical Computing, Vienna, Austria).

DIVERSITY OF VEGETATION

Within-field species richness

To evaluate plant community diversity within our field sites, we measured vascular plant species richness at each site in 2009. We counted plant species within concentric circles of 1, 10, and 100 m^2 at four locations within each field. Values for the four locations were then averaged for analyses. Although maize and switchgrass plantings were intended to be monocultures, volunteer species were also present.

Land cover and landscape diversity

We assessed land cover around each study site at 4 distances: 1.5, 5, 10, and 20 km. To do this, we employed two different data sources. To quantify vegetation in the more immediate landscape around each study site (1.5 km), we used georeferenced aerial imagery from the United States Department of Agriculture's 2009 National Agricultural Imagery Program (NAIP) orthoimagery (available at http://datagateway.nrcs.usda.gov), which has a grain size of 1 m^2. We established 1.5 km radius circles around each field site within which to determine land cover classes. Previous work has shown this distance to be appropriate for assessing landscape effects on insects, including parasitism of soybean aphids and parasitism of cereal aphids (Roschewitz et al. 2005, Gardiner et al. 2009a). The area within the 1.5 km radius circle was divided into polygons using ArcGIS 9.3 and manually assigned to land cover classes (Fig. 4.1b) by a trained aerial photography interpreter (R. Goodwin). The classification system included categories for agricultural grasses, pasture, manicured grasses, wild grasses, and mixtures of grasses with other wild vegetation, along with non-grass land cover types (Table B4.1).

To analyze broader areas around each site, we used the United States Department of Agriculture 2009 Cropland Data Layer (CDL; www.nass.usda.gov/research/Cropland). The CDL is created from imagery produced by the Advanced Wide Field Sensor (AWiFS) onboard the Resourcesat-1 satellite; its pixel size is 56 m x 56 m (Seffrin 2007), about 300 times greater than that of NAIP. This data set emphasizes agricultural crops (30 in our study area) and has fewer categories for herbaceous wild vegetation types (Table B4.2). Land cover classifications are made using a decision-tree classifier approach using images from multiple dates (USDA 2009). For agricultural classes, training data are derived from the Farm Service Agency's Common Land Unit, and the resulting classification accuracy is estimated to be ~90% (USDA 2009). For non-agricultural classes, training data is derived from the National Land-Cover Dataset (NLCD). The NLCD was developed for the U.S. federal government using Landsat 5 and 7 satellite imagery but has lower accuracy (~ 75%) (Homer et al. 2004). We assessed CDL land cover in circles with radii of 1.5, 5, 10, and 20 km around each of our field sites and around suction trap locations in Michigan and northern Indiana.

For both NAIP imagery and CDL analyses, we calculated percent cover of each land cover class around each study site. To assess the influence of broader land cover types, we combined some classes into more general categories (e.g., row crops, forest) (Appendices A & B). To quantify landscape diversity, we calculated Simpson's Diversity index (Simpson 1949), using the equation $D = 1/\Sigma(p_i)^2$, where p_i is the proportion of area in the *i*th land cover class (Gardiner et al. 2009b, Noma et al. 2010). We calculated Simpson's D both with all land cover classes included and with the exclusion of classes considered non-habitat (i.e., water and impervious surfaces). Because correlations were stronger when non-habitat cover classes were excluded, we present only that analysis here. To assess landscape patchiness, we calculated mean patch size of all polygons within the 1.5 km radius circle from NAIP photograph interpretation. The larger grain of the CDL results in salt-and-pepper effects (small scale intermixing of different classifications) and thus was not amenable to patch size calculations. To compare analysis based on CDL and NAIP interpretations, we used data from the 1.5 km radius circles to compare quantification of row crops, forest, wild vegetation, and landscape diversity.

WEATHER VARIABLES

For weather and climate information, we used data on precipitation and average maximum and minimum temperatures (Max T, Min T) from PRISM Climate Group (Oregon State University, http://www.prismclimate.org). PRISM (Parameter-elevation Regressions on Independent Slopes Model) uses point data and a digital elevation model to produce climate maps (Daly et al. 1993). For comparisons with spring aphid data collected in June 2009, we evaluated weather data from that month. For comparisons with late summer aphid data collected in August and September 2009, we used the averages of the maximum and minimum temperatures and the precipitation total for those two months. For comparisons with virus incidence data, we used annual climate normals (based on 1971-2000) because virus infection likely accumulated over several years.

ANALYSIS, MODEL SELECTION, AND SPATIAL AUTOCORRELATION

We conducted analysis of variance (ANOVA) to compare vector pressure among different community types and used linear regressions and correlations in R 2.11.1 to assess the influence of community, landscape, and environmental factors on virus and vector pressure. When multiple scales of data were available, such as for measures of species richness and landscape diversity, we compared the strength of the relationship with the response variables at all scales. We *ln*-transformed bowl trap and sticky trap aphid data to meet assumptions of normality. Pairwise comparisons for ANOVA used Holm-Bonferonni corrections for significance values.

To assess which variables or combinations of variables best described aphid pressure in biofuel crops, we used general linear models in R 2.11.1. For model selection, we calculated corrected Aikaike Information Criterion (AICc) values (Burnham and Anderson 2002). Models included species richness, land cover variables, and weather data as predictors. Models with the same response variable were compared using the difference between their AICc values (Δ_i). As a comparative or null model, we included field latitude as a predictor variable. Models with high multicollinearity (based on variance inflation factors) were dropped from analysis. To compare the influence of various predictor variables, we calculated standardized regression coefficients. For virus and suction trap data, correlation analysis was used instead of model selection due to the smaller number of data points ($n = 7$).

If significant autocorrelation had been found for measures of vector pressure, we then tested the residuals from the linear models (Diniz-Filho et al. 2003) found in model selection to determine whether predictor variables accounted for spatial autocorrelation.

Results

B/CYDV PREVALENCE

B/CYDV infection prevalence reached high levels in some switchgrass fields; natural B/CYDV infection rates ranged from 0–28%, as measured by diagnostic RT-PCR (Schrotenboer et al. 2011). Highest infection rates were found in two sites in neighboring counties (Kalamazoo and Barry counties) in southwestern Michigan (Fig. 4.2). Virus prevalence was not related to stand age among the five sites for which planting date was known ($R^2 = 0.27$, $P = 0.36$).

VECTOR MEASURES

Spring migration

The abundance of aphids caught in suction traps provides a synoptic assessment of aphid pressure across Michigan. Aphid abundance from suction traps showed strong seasonal fluctuations, with peak aphid flight in late spring and late summer/early fall (Fig. 4.3). The spring peak of migrating cereal aphids in Michigan was greater in 2009 than in 2008, but in both years greatest aphid pressure was in southwest Michigan (Fig. 4.4)—the same area where virus prevalence was greatest (Fig. 4.2). *Rhopalosiphum padi* was the most numerous cereal aphid—comprising 81–94% of cereal aphids during the 2008 and 2009 spring peaks in Michigan.

Field measures of alate pressure showed some similarities to numbers of migrating aphids caught in suction traps. As with suction trap data, alate pressure as measured by June card traps

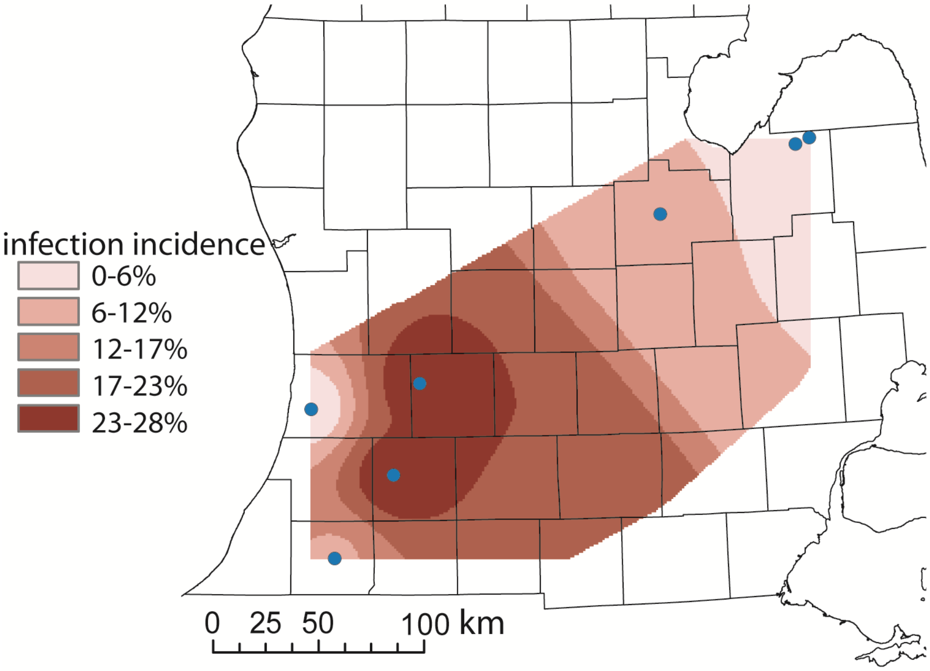

Fig. 4.2. B/CYDV prevalence in switchgrass fields sampled in 2008 as quantified with RT-PCR. Inverse distance weighting was used to interpolate between data points.

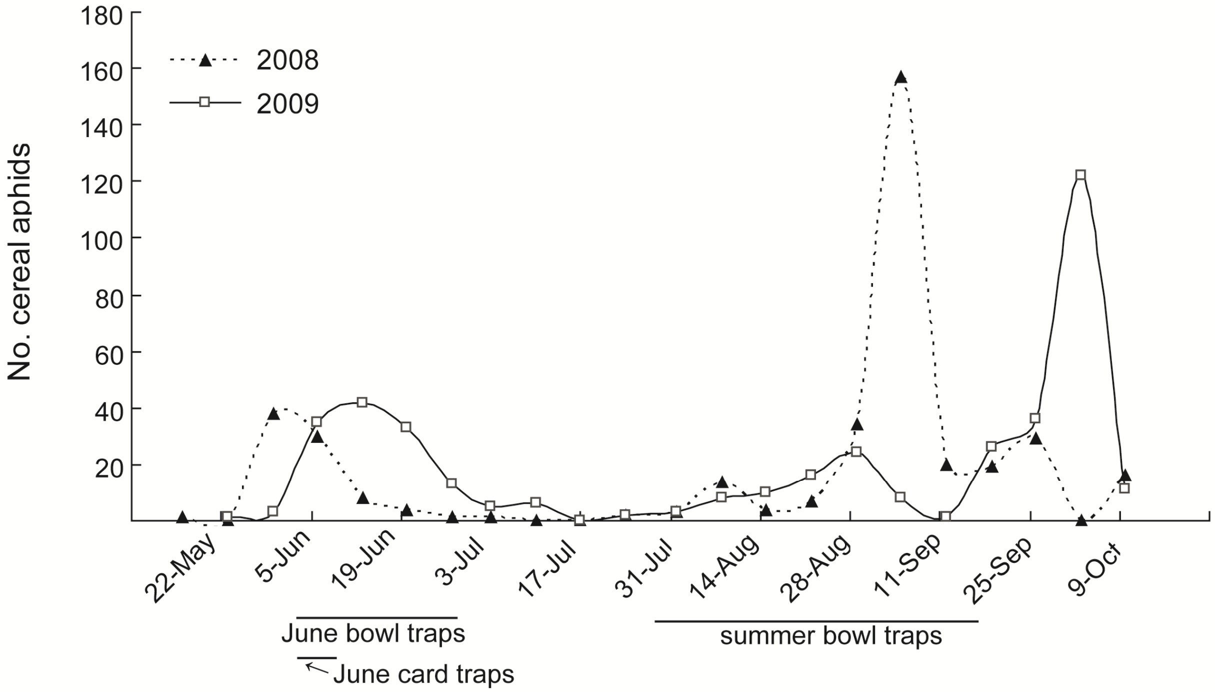

Fig. 4.3. Cereal aphids captured in suction traps in 2008 and 2009 in East Lansing, MI. Timing of 2009 bowl traps and sticky card traps relative to suction trap data is indicated.

was also highest in southwest Michigan in maize fields (Fig. 4.5a), in which aphids are expected to be largely arriving migrants. Among grassland sites where some carry-over of aphids from year to year may occur, alate numbers on card traps were greatest in southwest and south-central Michigan (Fig. 4.5b).

During the spring migration, sticky card traps caught more alates than did bowl traps. When values were standardized to account for differences in trap surface area, June bowl traps, which capture mainly down-welling alates, caught an average of 26.1 ± 5.8 alates wk^{-1} 0.1 m^{-2}. June sticky traps caught 96.3 ± 15 total alates wk^{-1} 0.1 m^{-2} and 92.2 ± 14.5 cereal alates wk^{-1} 0.1 m^{-2}.

During peak spring flight (June), bowl traps caught more alates in maize fields than in prairie sites (ANOVA, $F_{2,26} = 4.1$, $P = 0.03$) (Fig 6a), indicating greater numbers of incoming aphids into maize crops. Sticky card traps caught more alates in maize fields that in either prairie sites or switchgrass fields (ANOVA, $F_{2,15} = 3.8$, $P = 0.046$) (Fig. 4.6b).

Fall migration

The late summer/fall peak in migrating cereal aphids caught in suction traps occurred four weeks later in 2009 than 2008 (Fig. 4.3). Many fewer alates were caught in bowl traps in late summer (5 ± 0.5 aphids wk^{-1} 0.1 m^{-2}) than were caught in June. Low aphid numbers were likely due in part to the later peak in migrating cereal aphids in 2009 indicated by the suction trap data. During this period, community type had no evident effect on aphid numbers in bowl traps (ANOVA, $F_{2,26} = 0.3$, $P = 0.78$) (Fig. 4.6c).

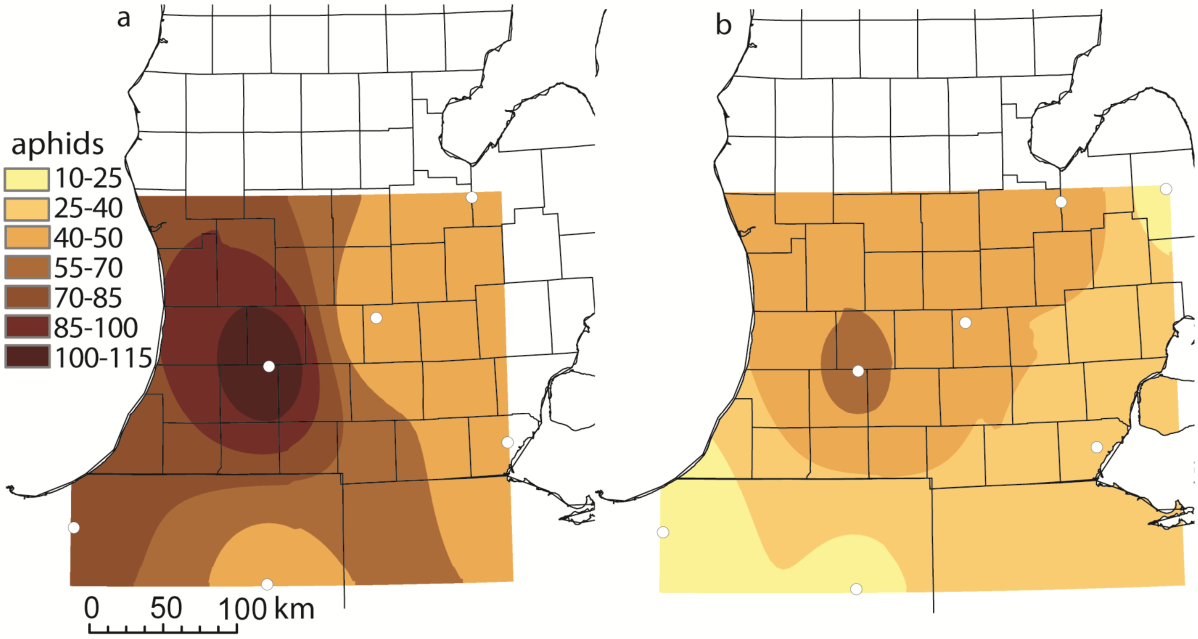

Fig. 4.4. Spring peak of cereal aphids caught in suctions traps in (a) 2008 and (b) 2009. Inverse distance weighting was used to interpolate between data points.

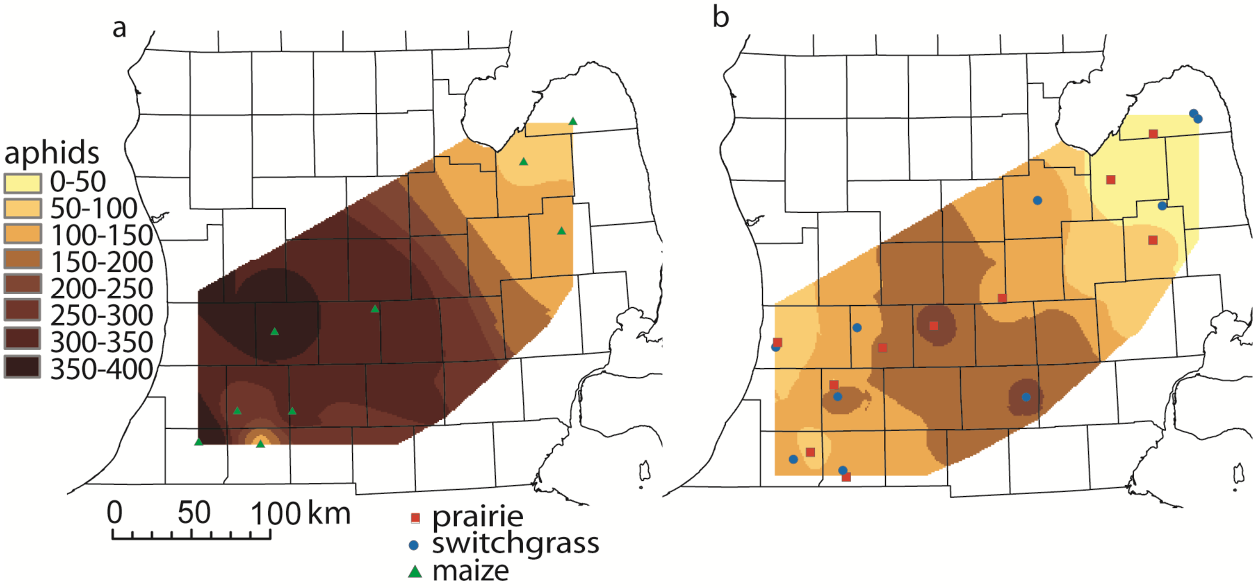

Fig. 4.5. Inverse distance weighting maps of aphids caught on June card traps in (a) maize and (b) grassland sites.

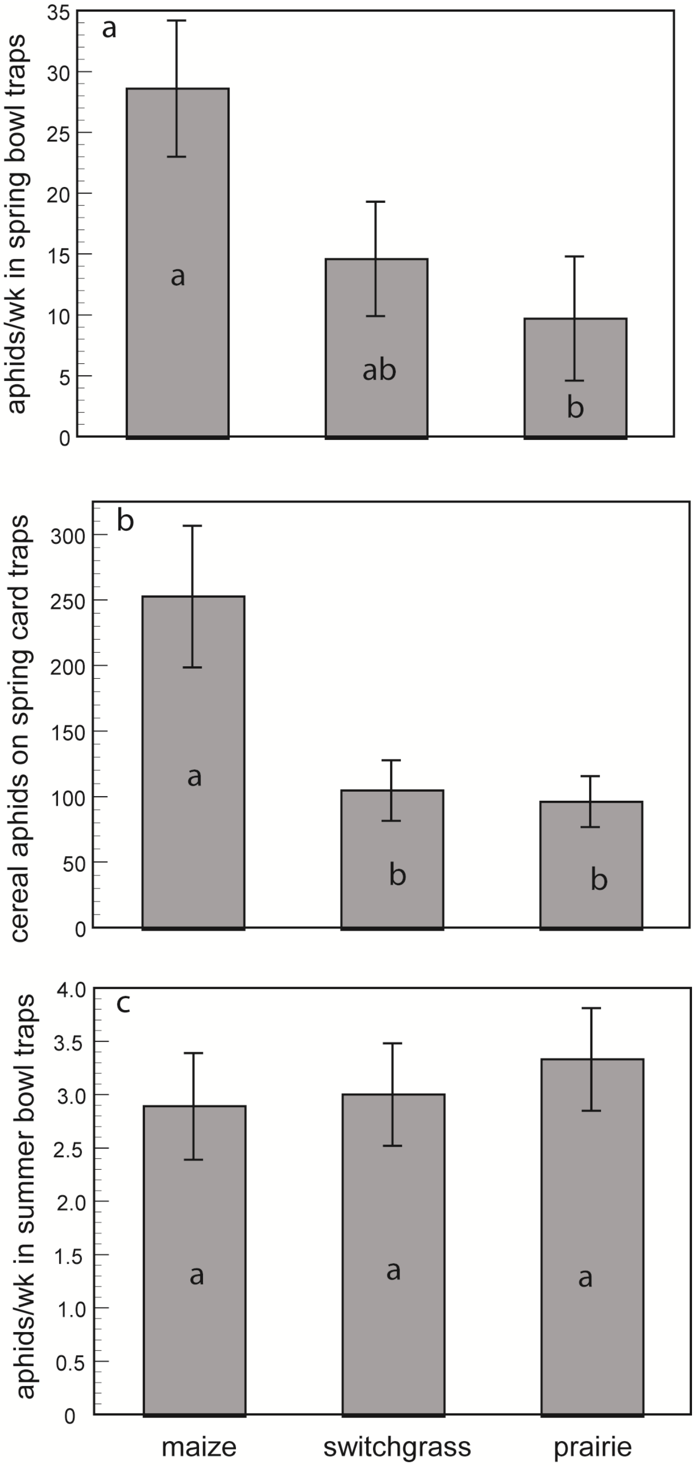

Fig. 4.6. Number of aphids caught according to community type in (a) June bowl traps, (b) June sticky card traps, and (c) late summer bowl traps. Different letters indicate significant differences.

Spatial patterns

Observed data from June card counts of cereal aphids were spatially autocorrelated (Moran's $I = 0.45$, $P = 0.0004$). However, data from bowl traps in June (Moran's $I = -0.11$, $P = 0.65$) and late summer (Moran's $I = 0.05$, $P = 0.53$) were not spatially autocorrelated.

COMPARISON OF LAND COVER ASSESSMENTS

In general, numerical estimates of land cover proportions derived from NAIP aerial photography and the Cropland Data Layer (CDL) were well correlated in the regions evaluated. The two methods showed similar forest land cover proportions ($R^2 = 0.95$, $P < 0.0001$) (Fig. 4.7a). The CDL slightly overestimated row crop cover and notably underestimated total wild vegetation cover in comparison with NAIP interpretation (Fig. 4.7b&c), although measures of both cover types were highly correlated between the two methods (row crops, $R^2 = 0.97$, $P < 0.0001$; wild vegetation, $R^2 = 0.90$, $P < 0.0001$). For land cover diversity measures, both methods were highly associated ($R^2 = 0.79$, $P < 0.0001$), but the CDL tended to overestimate landscape diversity for areas determined from NAIP interpretation to have intermediate diversity (Fig. 4.7d). Due to the concordance between measures for forest and row crops, we have high confidence in estimates of these cover types from the larger areas (e.g., 5, 10, and 20 km circles). Assessments of landscape diversity at these larger scales, however, may not be as representative due to differences in classification systems.

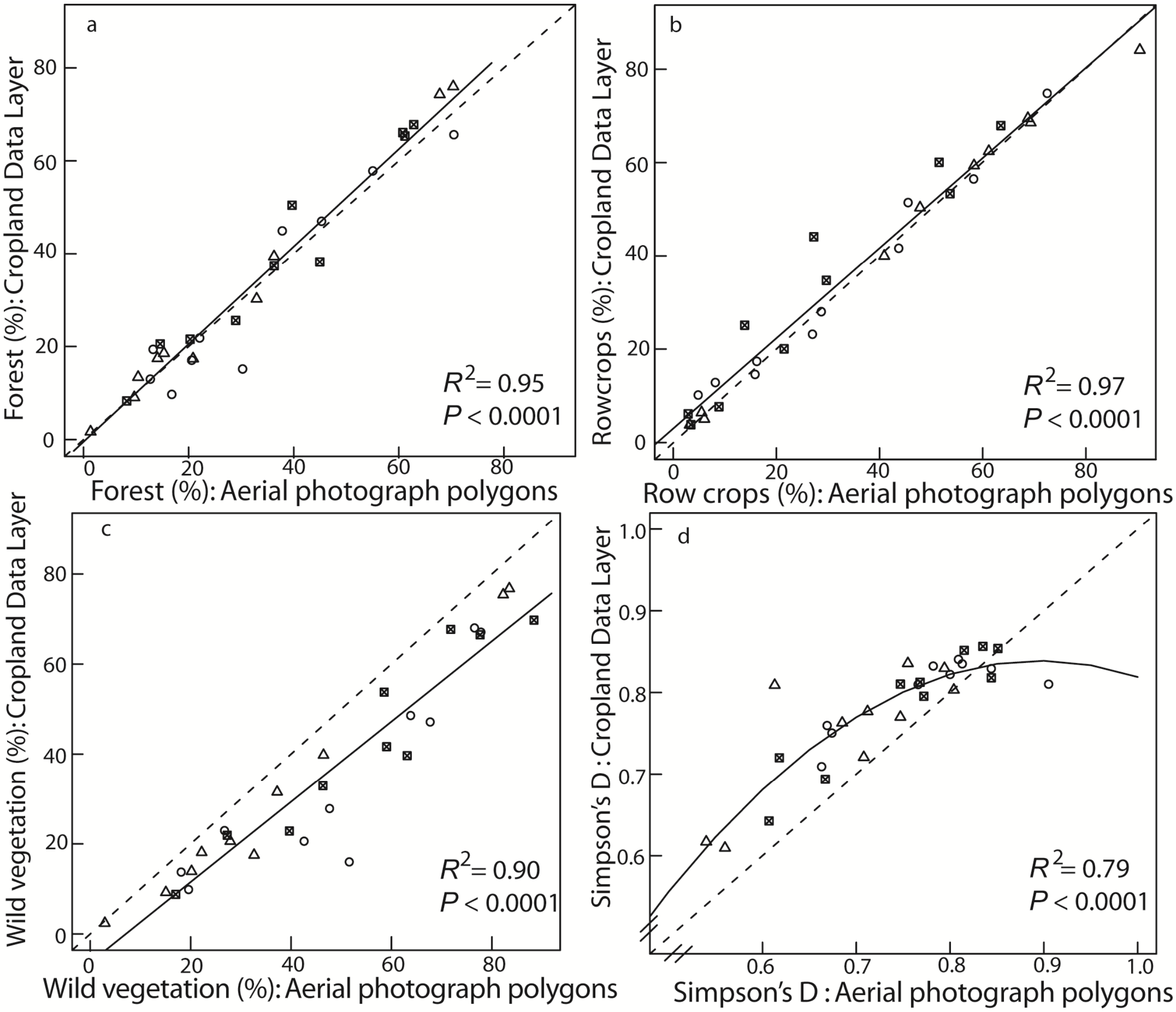

Fig. 4.7 Comparisons of NAIP aerial photograph interpretation and the Cropland Data Layer in estimates of (a) forest, (b) row crops, (c) wild vegetation, and (d) landscape diversity based on land cover within a 1.5 km radius of each study site (Δ maize, ○ switchgrass, □ prairie). Dotted lines represent a 1:1 relationship; solids lines are the fitted linear regression.

FACTORS INFLUENCING VECTOR PRESSURE

Model selection indicated that different processes and periods of aphid movement were sensitive to different influences; the three in-field measures of vector pressure—June bowl traps, June card traps, and late summer bowl traps—each had different best fit models (Table 4.1). However, land cover diversity was an important predictor of all three measures of in-field aphid pressure.

The best fit model (based on the lowest AICc value) for June bowl-trapped aphids incorporated landscape diversity (from aerial photography interpretation) and plant community richness and explained 69% of variation in the data (Table 4.1).

The best fit model for June card-trapped aphids incorporated Max T, plant community richness, and landscape diversity (from aerial photography interpretation) and explained 65% of variation in the data (Table 4.1). However, several other models were also highly supported ($\Delta_i < 2$). All four additional models included Max T and plant community richness, and landscape diversity or land cover components were also included in all but one supported model (Table 4.1). We found the best fit model reduced spatial autocorrelation (model residuals: Moran's *I* = 0.2, *P* = 0.053); the most complex supported model, even more so (model residuals: Moran's *I* = 0.08, *P* = 0.28). Thus, the predictors in our analysis are able to account for underlying spatial structure in the data.

For late summer aphid pressure, the best fit model included landscape diversity within 5 km and the percent of forested area in the surrounding 20 km (based on CDL data) although a simpler model incorporating only forest within 20 km was also supported ($\Delta_i < 2$) (Table 4.1). Other factors not investigated in this study likely contribute because the best fit model explained only 47% of variation in the data.

Table 4.1. Model selection indicates that landscape diversity, as measured by Simpson's D, was an important predictor of all three measures of in-field vector pressure. However, best fit models differed among the three aphid measures. LogLik = log likelihood; K_i = number of model parameters; AICc = corrected Akaike information criterion; ΔAICc = difference between AICc of best fit model and specified model.

Response variable	Model type	Model predictors	logLik	K_i	AICc	ΔAICc	Model R^2
June bowl traps	Best fit model	Simpson's D (1.5km NAIP[1]) + community richness[2]	-8.1	4	27.3	0.0	0.69
June card traps	Best fit model	Max T[3] + Simpson's D (1.5km NAIP) + community richness	-24.6	5	61.8	0.0	0.65
	Other supported models[4]	Max T + Simpson's D (1.5km NAIP) + community richness + row crops (1.5km NAIP)	-23.2	6	62.2	0.4	0.68
		Max T + Simpson's D (5km CDL[5]) + community richness	-24.9	5	62.4	0.6	0.64
		Max T + community richness + forest (20km CDL)	-24.9	5	62.4	0.6	0.64
		Max T + community richness	-26.6	4	62.9	1.1	0.64
Late summer bowl traps	Best fit model	Simpson's D (5km CDL) + forest extent (20km CDL)	-13.7	4	35.3	0.0	0.47
	Other supported model[4]	Forest extent (20km CDL)	-15.9	3	37.7	1.7	0.38

[1] National Agricultural Imagery Program, aerial photograph interpretation

[2] Community species richness within 1 m^2

[3] Average maximum temperature (from June to fit models of June aphid data)

[4] ΔAICc < 2

[5] Cropland Data Layer

In all cases, the null model, which included only latitude, had an AICc value at least 2 units greater than the best fit model. None of the landscape variables were significantly related to latitude (correlation, $P > 0.05$), indicating that land cover and landscape diversity differences are not an effect of latitude differences. However, as expected, several weather variables, including Max T, were related to latitude ($P < 0.05$).

EFFECTS OF PREDICTORS IN BEST FIT MODELS

For June bowl-trapped aphids, landscape diversity, as measured by Simpson's D, and community species richness were inversely related to vector pressure (Fig. 4.8a). However, the influence of landscape diversity was almost twice as great as that of within-field species richness (Table 4.2). Although plant species richness was included in the best fit model for aphids caught in June bowl traps, simple regression between these variables was not significant ($R^2 = 0.01$, $P = 0.66$) (Fig. 4.8a). However, in multiple regression with Simpson's D, the effect of species richness on June bowl traps was similar to that on June card traps (Table 4.2).

For June card-trapped aphids, Max T was most influential of the three variables included in the best fit model (Table 4.2). Greater average maximum temperatures in June were associated with greater numbers of aphids caught ($P < 0.0001$) (Fig. 4.8b). Both in-field plant community richness and landscape diversity were inversely related to vector pressure and had similar effect sizes (Table 4.2) (Fig. 4.8b). The inverse relationship with plant community richness was found at all three scales (1, 10, and 100 m^2) but was strongest at the scale of 1 m^2 (linear regression, $R^2 = 0.21$, $P = 0.01$).

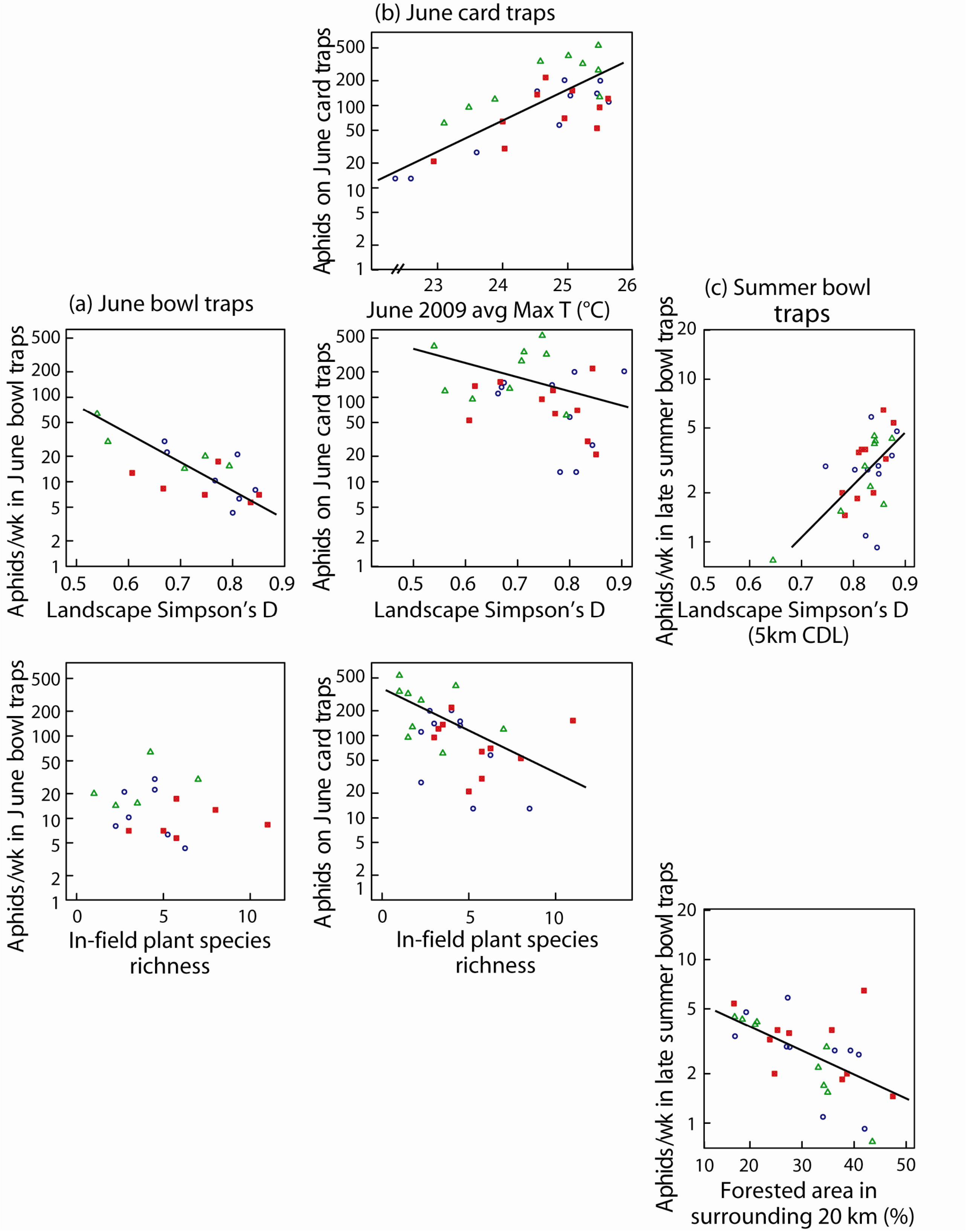

Fig. 4.8. Influence of variables identified in best fit models for predicting vector pressure from (a) June bowl traps, (b) June card traps, (c) late summer bowl traps.

Table 4.2. Relative influence of predictor variables identified in best fit models (Table 4.1). Standardized coefficients are calculated with all variables scaled to have a mean of 0 and standard deviation of 1. Comparison shows that landscape diversity (Simpson’s D) is more important than community richness in predicting June bowl-trapped aphids. The two variables had similar influence in predicting June card-trapped aphids.

Response variable	Predictor	Standardized coefficient
June bowl traps	Simpson's D (1.5km NAIP[1])	-0.87
	Community richness[2]	-0.48
June card traps	Max T[3]	0.58
	Community richness	-0.31
	Simpson's D (1.5km NAIP)	-0.24
Late summer bowl traps	Forest extent (20km CDL[4])	-0.44
	Simpson's D (5km CDL)	0.35

[1] National Agricultural Imagery Program, aerial photograph interpretation

[2] Plant community species richness within 1 m^2

[3] Average maximum temperature (from June to fit models of June aphid data)

[4] Cropland Data Layer

The response of aphids to landscape diversity differed later in the season; when aphids were beginning to seek winter hosts in late summer, more aphids were caught in fields in more diverse landscapes (Fig. 4.8c), which was the opposite effect of that seen in June. In addition, the greater the forested area in the surrounding landscape, the fewer aphids caught in bowl traps (Fig. 4.8c).

LANDSCAPE INFLUENCE ON APHIDS CAUGHT IN SUCTION TRAPS

Unlike other measures of vector pressure, spring peak aphid counts from suction traps were positively associated with CDL landscape diversity at 1.5 km ($r = 0.82$, $P = 0.02$) although not at larger scales (Table 4.3).

Spring aphid counts from suction traps were mostly strongly associated with specific land cover types at the largest area tested; correlations between alates and land cover were stronger when land cover was assessed within a 20 km radius than at any of the smaller distances (Table 4.3). The extent of row crops was inversely related to aphid counts; extent of forest was positively related (Table 4.3).

CONNECTION BETWEEN VIRUS AND VECTOR PRESSURE

Our initial studies suggest a relationship between virus and alate pressure. In the five switchgrass sites sampled both for virus in 2008 and for aphids in 2009, virus prevalence was generally greater in fields in which June bowl traps caught more aphids ($r = 0.82$, $P = 0.09$) (Fig. 4.9). Because this data is from different years, this suggests year-to-year consistency in vector and virus dispersal.

Table 4.3. Correlations between landscape factors assessed using Cropland Data Layer (CDL) data and the number of cereal aphids caught in suction traps in Michigan and northern Indiana during the spring peak of aphids.

		Spring suction trap aphids	
Landscape factor	Scale	r	*P*
Row crop extent	1.5 km	-0.57	0.18
	5 km	-0.55	0.20
	10 km	-0.53	0.22
	20 km	*-0.71*	*0.08*
Forest extent	1.5 km	0.58	0.16
	5 km	0.62	0.14
	10 km	0.59	0.16
	20 km	**0.76**	**0.05**
Simpson's D	1.5 km	**0.82**	**0.02**
	5 km	0.63	0.13
	10 km	0.59	0.16
	20 km	0.52	0.23

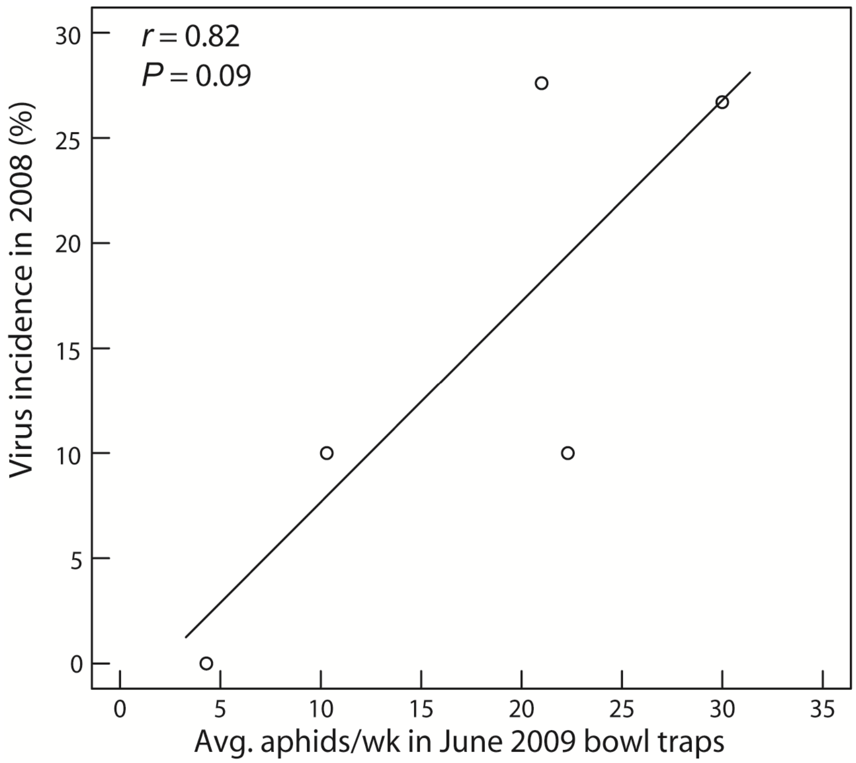

Fig. 4.9. Relationship between aphids trapped in switchgrass fields in 2009 and virus incidence in switchgrass plants sampled from those fields in 2008.

Table 4.4. Correlations between community, landscape, and weather factors and B/CYDV prevalence in switchgrass fields.

			B/CYDV incidence	
Factor type	Factor	Scale	r	*P*
Within-field	Community species richness[1]	1 m^2	-0.60	0.28
Landscape	Simpson's D	1.5 km (NAIP[2])	-0.47	0.29
		1.5 km (CDL[3])	-0.4	0.38
		5km (CDL)	**-0.89**	**0.007**
		10km (CDL)	-0.52	0.23
		20 km(CDL)	-0.24	0.61
	% Row crops	1.5 km (NAIP)	**-0.90**	**0.006**
		1.5 km (CDL)	**-0.89**	**0.007**
		5km (CDL)	**-0.86**	**0.01**
		10km (CDL)	**-0.75**	**0.05**
		20 km(CDL)	-0.55	0.20
	% Forest	1.5 km (NAIP)	*0.70*	*0.08*
		1.5 km (CDL)	0.52	0.23
		5km (CDL)	0.5	0.25
		10km (CDL)	0.43	0.34
		20 km(CDL)	0.52	0.24
	% Grass habitat	1.5 km (NAIP)	-0.60	0.15
	Mean patch size	1.5 km (NAIP)	-0.17	0.72
Weather[4]	Precipitation		0.24	0.61
	Max T		0.65	0.11
	Min T		0.21	0.66

[1] Data only available for 5 out of 7 sites

[2] National Agricultural Imagery Program, aerial photograph interpretation

[3] Cropland Data Layer

[4] From June PRISM data

An assessment of how virus prevalence is related to community composition, landscape patterns, and weather factors shows that infection rates may not respond in exactly the same manner as vector pressure. Most notably, B/CYDV prevalence in switchgrass fields was inversely related to the row crop extent within 1.5, 5 and 10 km radii (Table 4.4), whereas extent of row crops was not included in best fit models of vector pressure. However, landscape diversity had a significant effect on both vector numbers and virus incidence; B/CYDV prevalence was inversely related to landscape diversity within 5 km, although this effect was not evident at any other scales (Table 4.4). B/CYDV prevalence was not associated with community species richness or temperature (Table 4.4), which had been important in predicting spring vector pressure (Table 4.1).

Discussion

To meet demands for bioenergy production, changes in landscape composition and agricultural crops are required (Perlack et al. 2005, Field et al. 2008). While these modifications will be highly visible, they can lead to changes in virus and vector dynamics, which although less readily apparent, are equally important to consider when examining potential repercussions of biofuel production. The ecology of plant viruses in native perennials has been given little attention compared to infection in annual crops. Now, with the advent of perennial biofuel cropping systems, interested growers and researchers have realized that knowledge of virus interactions with these novel crops—so recently moved from native prairies into domestication—is highly limited but potentially critical (Agindotan et al. 2010, Landis and Werling 2010). In the Poaceae-dominated systems of the Midwest, changes in virus dynamics among generalist, aphid-vectored viruses like B/CYDVs have the potential to influence not only biofuel crops, but also cereal grains and native prairies. A key mechanism through which biofuel-driven changes may influence virus severity and distribution is through effects on vector pressure.

Our analysis demonstrates that in this system landscape diversity is one of the factors most strongly linked to vector pressure, with additional effects related to local community richness and temperature. This new finding is consistent with previous work in which landscape diversity has been linked to abundance of natural enemies (Bianchi et al. 2006, Landis et al. 2008, Gardiner et al. 2009a). Our study also reveals that landscape diversity effects can be seasonally dependent, thus highlighting the need to develop mechanistic understanding of diversity influences on virus systems.

LANDSCAPE INFLUENCE

When migrating aphids were seeking hosts in spring, vector pressure was greatest in less diverse landscapes. However, at the start of the fall migration, the relationship was reversed, and more aphids were caught in diverse landscapes. The timing of B/CYDV infection can affect disease severity (Perry et al. 2000), and thus, seasonal differences in the influence of landscape diversity are important for understanding virus activity among biofuel crops and surrounding vegetation.

The spring relationship we observed is consistent with the findings of Gardiner et al. (2009a) who found that natural biocontrol of soybean aphids increased with landscape diversity. Natural enemies are known to be effective control agents against cereal aphids in winter wheat (Chambers et al. 1986), so the reduction in cereal aphid pressure we observed in diverse landscapes may have resulted from increased biocontrol. In contrast, in Germany, Roschewitz et al (2005) observed a positive relationship between landscape complexity and cereal aphid numbers in spring and early summer. However, their measure of landscape complexity differed notably from our diversity calculation; in the German study, complex landscapes were defined as those in which annual row crops comprised < 65% of total land cover, whereas we calculated a Simpson's D from land cover types, which included different types of agricultural crops. These methodological differences may partly explain the contrasting results.

While landscape diversity was the most influential factor across measurements of vector pressure, the extent of particular land cover types was also associated with vector and virus prevalence in our study. Our findings indicate a need to investigate how particular land cover classes exert influence on vector pressure, such as by dampening aphid activity in biofuel fields. For example, one such mechanism may involve the presence of alternative hosts; forest provides winter habitat for host-alternating aphids such as *R. padi*, which rely on Poaceae hosts in summer and woody hosts, such as wild *Prunus* species, in winter (Dixon 1971, Vickerman and Wratten 1979). Thus, the attractiveness of forest to *R. padi* during fall migration could explain why vector pressure was lower in fields with greater forested land cover in the surrounding area, as aphids moved out of herbaceous vegetation and into forested areas. Pesticide-treated vegetation types may act as sinks if aphids are attracted to them but are killed. Cereal crops and turf grasses are often treated with neonicotinoids, such as thiamethoxam and imidacloprid, either as seed treatments or foliar applications (Elbert et al. 2008). When applied as seed treatments, these systemic insecticides protect juvenile plants, on which cereal aphids have limited survival and reproduction (Gray et al. 1996). As a result, aphid numbers may fall when spring migrants land in treated cereal crops; virus incidence thereby may be reduced as well, which is consistent with our finding that virus prevalence was inversely related to the percentage cover of row crops in the surrounding landscape.

INFLUENCE OF COMMUNITY COMPOSITION

In addition to landscape context, species composition of biofuel crops can also influence vector pressure during spring alate migration. In our temperate study system, we found that species-diverse grassland sites experienced less vector pressure in spring than species-depauperate maize fields, indicating that perennial polyculture could contribute to pest control in biofuel systems.

We found that plant community species richness was an important factor for predicting spring vector pressure. Borer et al. (2010) also found community composition to be an important factor influencing B/CYDV prevalence in a Mediterranean-type system. However, in their annual-dominated system, cover of perennial grasses was found to be a critical controlling factor, rather than host species richness. In addition, the authors concluded that community context was the most important driver of virus dynamics (Borer et al. 2010), whereas in our temperate system, we found landscape patterns to have stronger and more widespread effects on vector pressure.

Vegetation attractiveness to alates is related to visual cues of color and reflectance (Qualset et al. 1990). Row crops provide a combination of vegetation and exposed ground, which can attract some aphid species (Costello 1995), and which may partly explain why more alates were trapped in maize fields than in perennial grass stands.

The perenniality of grassland crops is also important to consider when evaluating vector and virus dynamics. Annual crop fields provide little insect carryover from year to year, but perennial systems, such as switchgrass and prairie, may allow natural enemies to overwinter (Landis et al. 2000). However, some cereal aphids that utilize grass host species throughout the year (e.g., *Sitobion avenae*) also may overwinter in perennial grasses (Vickerman and Wratten 1979). In our data, aphid pressure in maize fields, which are planted anew each year and thus have little carryover between years, were most similar to regional patterns of aphid pressure measured by suction traps, which capture long distance migrants. Patterns among grassland sites were more complex, indicating the influence of overwintering natural enemies and aphids.

Another area for future investigation is to what extent community composition influences the export of vectors and virus. Do certain biofuel crops amplify vector populations that then spill over into the landscape? Which factors are most important in determining virus export?

ENVIRONMENTAL FACTORS

Warmer spring temperatures contributed to greater alate pressure within biofuel crop fields. Temperature is known to be positively associated with aphid developmental rates and population growth rates up to a threshold beyond which rates decline (Dean 1974, Carter 1985). For example, *R. padi* had maximum rates of increase at 25°C under laboratory conditions (Dean 1974), and June 2009 maximum temperatures ranged from 22.4–25.6°C. Aphids in areas of higher temperatures may have experienced more local population growth than those in cooler areas, contributing to the numbers of aphids caught on card traps.

This environmental effect suggests that climate warming could increase vector pressure and allow vector numbers to increase earlier in the season. Climate change is recognized as a driving force in the emergence and increased severity of infectious diseases, which could threaten both cultivated crops and wild plants (Anderson et al. 2004, Jones 2009), and B/CYDVs and other aphid-transmitted pathogens are likely to be in the group of pathogens affected.

IMPLICATIONS

As bioenergy production expands, biofuel crops will not only be affected by landscape context but also will influence landscape diversity and homogeneity (Dauber et al. 2010), with attendant effects on aphid and virus prevalence. To mitigate potential negative outcomes for biofuel crop productivity, choices about crop diversity and landscape location can be made to minimize risks of vector and virus pressure. For example, biorefineries that accept multiple feedstocks could help create more diverse landscapes (Landis et al. 2008). As biofuel crops become a larger portion of the landscape, potential spillover effects from these new crops into other vegetation types also need to be considered. Our study suggests that more homogeneous landscapes could increase vector pressure on neighboring cereal crops due to reduced landscape diversity (although the intensity of the change might depend on the particular biofuel species planted), and more diverse landscapes could decrease vector pressure. Effects of diversity on viruses are likely to follow those of their aphid vectors to a large extent. Understanding the controlling factors for different seasons and types of vector movement will allow future work to better model the mechanisms of vector and B/CYDV dispersal and predict how future land use changes will alter pathogen dynamics.

Careful planning of biofuel landscapes could mitigate negative interactions with pests and pathogens. Choices may occur on multiple levels—from growers deciding to plant a diverse crop as opposed to a monoculture, to policies that provide incentives for diversifying agricultural landscapes. The potential for pathogens in biofuel crops to spill over into other systems, such as food agriculture and conservation areas, highlights the need for assessing such externalities and developing landscape-scale plans for bioenergy development.

CHAPTER 5

CONCLUSIONS

Humans have exerted considerable selection pressure on native prairie grasses. This was evident in the plastic response to fertile soils among propagated populations of *Andropogon gerardii* and in more biofuel-valuable traits in selected cultivars of *Panicum virgatum*. Such trait changes are likely to become more notable as these species are further modified for use as biofuel crops.

My work highlights the need to understand how trait changes in native species influence their ecological interactions. One consequence of such trait modifications could be altered community dynamics in prairie ecosystems—either through direct planting of propagated genotypes or through gene flow into remnant populations. My study of competition with *A. gerardii* suggests that propagated populations, despite their potential for higher productivity on fertile soils, did not out-compete wild-collected genotypes or a prairie forb. However, future changes to native species may be more extreme, including genetic transformations, which could create more significant competitive effects.

The potential for interactions of *Barley and cereal yellow dwarf viruses* (B/CYDVs) with switchgrass biofuel crops is evident. The next step is to investigate how infection with B/CYDVs and other viruses affect fitness of native prairie grasses. Based on interactions between B/CYDVs and other perennial grasses (Malmstrom et al. 2005a, Malmstrom et al. 2006), infection is expected to reduce fitness and productivity, thereby potentially decreasing biofuel feedstock yields. However, our understanding of pathogen interactions with native species is quite limited; in some cases, viruses can act as mutualists, particularly under stressful conditions (Gibbs 1980, Xu et al. 2008). If B/CYDVs do have negative fitness effects on switchgrass, can these be mitigated through breeding? Or should transgenic resistant lines be created? Alternatively, could we harness potential mutualistic associations with viruses to improve biofuel crop production on marginal lands?

To understand the broader landscape implications of virus–biofuel interactions, we also need to better understand the dynamics of aphid population growth and aphid movement from biofuel crops into other vegetation. We have now identified several landscape and community factors than influence aphid abundance in biofuel crops, but how long do aphids remain in these fields? Duration of feeding is an important determinant of virus acquisition and transmission (Gray et al. 1991, Power et al. 1991). The potential for virus spillover into other vegetation likely is greatest if aphids feed long enough to transmit or acquire virus in a biofuel crop but then move on to find more preferable hosts. If diverse biofuel crops also promote natural enemies, does this reduce aphid pressure and thereby dampen virus spread?

Avenues of inquiry into the effects of human influences on natural systems and native species are many. Key considerations in future work will be determining which potential consequences may have the possibility of undesirable repercussions. Changes to landscape pathogen dynamics stand out in this respect because of implications for both human-managed and natural systems.

APPENDICES

APPENDIX A

Table A2.1. Results of ANOVA for measures of growth and reproductive investment in *A. gerardii*. Type indicates either propagated or wild-collected populations.

Year	Trait	Factor	*df*	*F*	*P*
Yr 1	Height	Type	1, 5	12.7	**0.02**
		Population (type)	4,4	7.6	**0.04**
		Soil	1, 2	3.6	0.22
		Type*soil	1, 4	17	**0.01**
		Soil*population(type)	4, 508	0.6	0.64
		Location	1, 1	0.02	0.9
		Soil*location	1, 91	4.2	**0.04**
	Tillers	Type	1, 6	0.4	0.58
		Population (type)	4, 4	10.5	**0.03**
		Soil	1, 6	5.7	**0.05**
		Type*soil	1, 6	10.4	**0.02**
		Soil*population(type)	4, 508	0.7	0.61
		Location	1, 1	0.1	0.86
		Soil*location	1, 91	1.9	0.18
	No. inflorescences	Type	1, 5	2.3	0.19
		Population (type)	4, 4	0.5	0.75
		Soil	1, 1	5.1	0.27
		Type*soil	1, 5	4.8	**0.01**
		Soil*population(type)	4, 344	0.6	0.66
		Location	1, 1	1.7	0.42
		Soil*location	1, 60	3.8	**0.05**
	Seedhead mass	Type	1, 7	2.4	0.17
		Population (type)	4, 4	0.9	0.56
		Soil	1, 1	22.19	0.13
		Type*soil	1, 7	7.7	**0.03**
		Soil*population(type)	4, 496	1	0.39
		Location	1, 1	3.4	0.32
		Soil*location	1, 92	2.0	0.16
	Total aboveground biomass	Type	1, 6	28.3	**0.002**
		Population (type)	4, 4	2.5	0.20
		Soil	1, 1	13.3	0.17
		Type*soil	1, 5	29.4	**0.002**
		Soil*population(type)	4, 494	0.8	0.52
		Location	1, 1	0.2	0.73
		Soil*location	1, 92	4.9	**0.03**

Table A2.1 (cont'd)

Year	Trait	Factor	*df*	*F*	*P*
Yr 2	Height	Type	1, 5	4.7	**0.09**
		Population (type)	4, 4	6.4	**0.05**
		soil	1, 1	3	0.33
		Type*soil	1, 4	4	0.37
		Soil*population(type)	4, 441	1.2	0.3
		Location	1, 1	19.9	**0.14**
		Soil*location	1, 78	0.8	0.38
	Basal area	Type	1, 4	0.02	0.91
		Population (type)	4, 4	23.4	**0.005**
		Soil	1, 1	6	0.25
		Type*soil	1, 6	5.6	*0.06*
		Soil*population(type)	4, 440	0.3	0.87
		Location	1, 1	1.6	0.43
		Soil*location	1, 78	0.8	0.38
	No. inflorescences	Type	1, 4	16.6	**0.02**
		Population (type)	4, 4	1	0.51
		Soil	1, 1	7.1	0.20
		Type*soil	1, 4	4.7	*0.10*
		Soil*population(type)	4, 283	1.6	0.16
		Location	1, 1	0.2	0.76
		Soil*location	1, 49	6.1	**0.02**
	Total aboveground biomass	Type	1, 4	30.3	**0.004**
		Population (type)	4, 4	2.7	0.18
		Soil	1, 1	20.5	0.14
		Type*soil	1, 5	14.1	**0.01**
		Soil*population(type)	4, 416	0.7	0.59
		Location	1, 1	2.5	0.36
		Soil*location	1, 74	1.7	0.20
	Avg seed weight	Type	1, 6	4.7	*0.07*
		Population (type)	4, 4	4.6	*0.08*
		Soil	1, 1	2.6	0.35
		Type*soil	1, 5	0.6	0.46
		Soil*population(type)	4, 409	0.9	0.48
		Location	1, 1	2.9	0.34
		Soil*location	1, 76	13.5	**0.0004**

Table A2.1 (cont'd)

Year	Trait	Factor	*df*	*F*	*P*
Yr 4	Height	Type	1, 4	0.3	0.61
		Population (type)	4, 4	9.6	**0.03**
		Soil	1, 1	10.4	0.19
		Type*soil	1, 6	19.1	**0.005**
		Soil*population(type)	4, 171	0.8	0.53
		Location	1, 1	1.4	0.45
		Soil*location	1, 29	2.9	*0.10*
	Basal area	Type	1, 4	0.1	0.94
		Population (type)	4, 4	11.4	**0.02**
		Soil	1, 1	3.9	0.30
		Type*soil	1, 4	0.4	0.57
		Soil*population(type)	4, 171	0.5	0.71
		Location	1, 1	17.1	0.19
		Soil*location	1, 30	0.1	0.77
	Total aboveground biomass	Type	1, 4	12.3	**0.02**
		Population (type)	4, 4	0.9	0.53
		Soil	1, 1	2.6	0.35
		Type*soil	1, 4	4.6	*0.10*
		Soil*population(type)	4, 171	1.2	0.30
		Location	1, 1	0.1	0.86
		Soil*location	1, 30	2.3	0.14

Table A2.2. Results of ANOVA for measures of growth and reproductive investment in *S. scoparium*. Type indicates either propagated or wild-collected populations.

Year	Trait	Factor	*df*	*F*	*P*
Yr 1	Height	Type	1, 3	3.6	0.16
		Population (type)	3, 3	25.2	**0.01**
		Soil	1, 1	0.8	0.53
		Type*soil	1, 3	0.7	0.47
		Soil*population(type)	3, 372	0.9	0.46
		Location	1, 1	1	0.50
		Soil*location	1, 94	0.21	0.65
	Basal area	Type	1, 3	0.0	0.98
		Population (type)	3, 3	26.5	**0.01**
		Soil	1, 1	0.1	0.98
		Type*soil	1, 3	0.2	0.66
		Soil*population(type)	3, 371	1.6	0.20
		Location	1, 1	0.6	0.58
		Soil*location	1, 93	8.5	**0.005**
	No. inflorescences	Type	1, 3	3.1	0.18
		Population (type)	3, 3	24.5	**0.01**
		Soil	1, 1	0.1	0.82
		Type*soil	1, 3	0.2	0.73
		Soil*population(type)	3, 235	1.1	0.35
		Location	1, 1	0.94	0.51
		Soil*location	1, 61	8.6	**0.005**

Table A2.2 (cont'd)

Year	Trait	Factor	*df*	*F*	*P*
Yr 2	Height	Type	1, 2	0.5	0.56
		Population (type)	3, 3	16	**0.02**
		Soil	1, 1	2.1	0.38
		Type*soil	1, 1	1.1	0.49
		Soil*population(type)	3, 306	0.9	0.42
		Location	1, 1	5.3	0.26
		Soil*location	1, 82	6.4	**0.01**
	Basal area	Type	1, 3	0.1	0.75
		Population (type)	3, 3	33.4	**0.008**
		Soil	1, 1	5.0	0.27
		Type*soil	1, 2	0.1	0.81
		Soil*population(type)	3, 302	1.1	0.34
		Location	1, 1	0.8	0.54
		Soil*location	1, 82	10.1	**0.002**
	No. inflorescences	Type	1, 3	1.6	0.30
		Population (type)	3, 3	2.2	0.27
		Soil	1, 1	1.5	0.40
		Type*soil	1, 1	0.2	0.74
		Soil*population(type)	3, 184	1.0	0.41
		Location	1, 1	0.5	0.62
		Soil*location	1, 54	18.6	**<0.0001**
	Total aboveground biomass	Type	1, 3	1.4	0.33
		Population (type)	3, 3	4.9	0.11
		Soil	1, 1	2	0.4
		Type*soil	1, 2	0.1	0.75
		Soil*population(type)	3, 288	4.2	**0.007**
		Location	1, 1	0.3	0.68
		Soil*location	1, 78	23.9	**<0.0001**
	Avg seed weight	Type	1, 3	0.0	0.99
		Population (type)	3, 3	160.3	**0.0008**
		Soil	1, 1	17.4	0.15
		Type*soil	1, 1	0.8	0.4
		Soil*population(type)	3, 275	0.5	0.68
		Location	1, 1	15.9	0.16
		Soil*location	1, 84	0.5	0.48

Table A2.2 (cont'd)

Year	Trait	Factor	*df*	*F*	*P*
Yr 4	Height	Type	1, 3	1.7	0.27
		Population (type)	3, 3	3.6	0.16
		Soil	1, 1	0.4	0.66
		Type*soil	1, 4	0.7	0.47
		Soil*population(type)	3, 113	1.1	0.34
		Location	1, 1	3.5	0.31
		Soil*location	1, 31	3	*0.09*
	Basal area	Type	1, 3	0.1	0.77
		Population (type)	3, 3	347.1	**0.0003**
		Soil	1, 1	54.6	*0.09*
		Type*soil	1, 3	105.6	**0.002**
		Soil*population(type)	3, 113	0.1	0.98
		Location	1, 1	0.7	0.55
		Soil*location	1, 32	1.6	0.21
	Total aboveground biomass	Type	1, 3	0.0	0.89
		Population (type)	3, 3	11.4	**0.04**
		Soil	1, 1	2.8	0.34
		Type*soil	1, 3	1.2	0.36
		Soil*population(type)	3, 113	1.5	0.23
		Location	1, 1	0.0	0.99
		Soil*location	1, 32	8.4	**0.007**

Table A2.3. Results of the Gower similarity index comparing soil characteristics of planting sites and source locations. The index can range from 0 to 1, with lower numbers indicating greater similarity.

Population	EL loam	Mason loam	EL sand	Mason sand
Oakland	0.323	0.661	0.382	0.331
Washtenaw	0.381	0.633	0.237	0.374
Kalamazoo	**0.297**	**0.488**	0.227	0.343
Berrien	0.403	0.620	**0.136**	0.190
St. Joseph	0.326	0.639	0.234	**0.153**

APPENDIX B

Table B4.1. Land cover classes used for interpretation of NAIP aerial photographs. Both grasses and cereal crops were included in grass land cover; cereal crops and additional crops were included in row crop cover; non-vegetated classes were not included in Simpson's D calculations.

Land cover type	Land cover class	Additional description
Forest	Deciduous forest	> 50% deciduous canopy forest
	Coniferous forest	< 50% deciduous canopy forest
Grasses	Developed grassland 75-100%	> 75 % manicured grasses (lawns, golf courses, etc.)
	Developed grassland 50-75%	50-75% manicured grasses
	Developed grassland 25-50%	25-50% manicured grasses
	Wild-natural grasses	Dominated by wild grasses
	Grass-shrub mix	> 25% grassland, > 25% shrub cover, < 25% tree cover
	Grass-forest mix	> 25% grassland, < 50% tree cover
	Pasture	Primarily grasses, grazed by livestock
Cereal crops	Small grains	Small grain crops such as wheat and barley
	Corn-sorghum	
Additional crops	Soybeans	
	Other row crops	Row crops other than those specified separately
Additional wild vegetation	Emergent wetlands	> 50% emergent wetland vegetation
	Shrub wetlands	> 50% wetland shrubs
	Forage-hay	Legumes, grasses, or a mix harvested for animal feed
Non-vegetated	Hardscape-impervious	> 75% impervious surfaces
	Water	Ponds, lakes, and rivers

Table B4.2. Land cover classifications used in the Cropland Data Layer (CDL) that were within 20 km of study sites. Non-vegetated classes were not included in Simpson's D calculations.

Land cover type	Land cover class	Further description
Forest	Deciduous forest	> 75% deciduous canopy forest
	Evergreen forest	> 75% evergreen canopy forest
	Mixed forest	25-50% deciduous canopy forest
	Woody wetlands	> 20% forest or shrubs, covered or saturated with water periodically
Row crops	Corn	
	Sweet corn	
	Other corn	
	Sorghum	
	Soybeans	
	Sunflowers	
	Barley	
	Winter wheat	
	Winter wheat, soybeans	Double cropped
	Rye	
	Oats	
	Speltz	
	Other small grains	
	Sugar beets	
	Dry beans	
	Potatoes	
	Onions	
	Peas	
	Other fruits and vegetables	
	Other crops	
	Herbs	
	Idle cropland	

Table B4.2 (cont'd)

Land cover type	Land cover class	Further description
Other vegetated cover	Alfalfa	
	Other hay	
	Clover/wildflowers	
	Pasture	
	Cherry	
	Apples	
	Grapes	
	Christmas trees	
	Barren	< 15% vegetation cover
	Shrubland	Dominated by shrubs less than 5 m tall
	Grassland	> 80% herbaceous vegetation not intensively managed
	Herb wetlands	> 80% herbaceous vegetation, covered or saturated with water periodically
	Developed open	Impervious surfaces < 20%, mostly lawn grasses
	Developed low	Impervious surfaces 20-49%
	Developed medium	Impervious surfaces 50-79%
Non-vegetated classes	Developed high	Impervious surfaces 80-100%
	Water	

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