

Spatial and temporal dynamics of *Puccinia andropogonis* on *Comandra umbellata* and *Andropogon gerardii* in a native prairie

Charles W. Barnes, Linda L. Kinkel, and James V. Groth

Abstract: The rust *Puccinia andropogonis* (Schwein.) was studied on its aecial and telial plant hosts, comandra (*Comandra umbellata* L. Nutt.) and big bluestem (*Andropogon gerardii* Vitman), respectively, in a native prairie to investigate the influence of aecial host proximity on disease severity of the telial host in a natural system. Both hosts were mapped to measure distances from comandra clones to selected big bluestem plants in a Minnesota prairie. Mean rust severity on big bluestem was regressed on the number of aecia on comandra (aecial density) within eight distance intervals from big bluestem plants. Distance intervals were analyzed both inclusive and exclusive of other distance intervals. There was a significant positive relationship between aecial density on comandra and rust severity on big bluestem that decreased with increasing distance in accordance with the power law model, becoming nonsignificant at distances >40 m. To establish whether the genetic background of big bluestem influenced the relationship between rust severity and the distance to comandra, a common garden was planted with a representative big bluestem plant population. The low coefficient of determination (r^2) between mean rust severities of individual plants from one year to another suggests there is not a strong genetic component in the host determining disease severity.

Key words: disease severity, naturally occurring rust, tallgrass prairie.

Résumé : Les auteurs ont étudié la rouille *Puccinia andropogonis* (Schwein.), chez ses hôtes portant les écidies et les télies, soit, respectivement, la comandre (*Comandra umbellata* L. Nutt.) et le barbon de Gérard (*Andropogon gerardii* Vitman) dans une prairie vierge. Le but était d'étudier l'influence de la proximité de l'hôte portant les écidies sur la sévérité de la maladie chez l'hôte portant les télies, dans un milieu naturel. Ils ont cartographié les deux hôtes en mesurant les distances entre les clones de la comandre, par rapport à des plants sélectionnés de barbans, dans une prairie au Minnesota. La sévérité moyenne de la rouille sur les barbans a été reliée, par régression, avec le nombre d'écidies sur les comandres (densité des écidies), à l'intérieur de huit intervalles de distance par rapport aux barbans. Ils ont conduit l'analyse des intervalles de distance, à la fois inclusivement et exclusivement des autres intervalles de distance. On retrouve une relation positive significative, entre la densité des écidies sur la comandre, et la sévérité de la rouille sur le barbon, laquelle diminue avec une augmentation de la distance, en concordance avec le modèle du « power-law », devenant non significative aux distances >40 m. Afin d'établir si la base génétique du barbon influence la relation entre la sévérité de la rouille et la distance par rapport à la comandre, les auteurs ont constitué un jardin commun, avec une population représentative du barbon. Le faible coefficient de détermination (r^2) entre les sévérités moyennes de la rouille chez des plantes individuelles, d'une année à l'autre, suggère qu'il n'y a pas de composante génétique dans le déterminisme de la sévérité de la maladie chez l'hôte.

Mots clés : sévérité de la maladie, rouille d'incidence naturelle, prairie d'herbes hautes.

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Introduction

It is generally thought that spatial structure of host populations is likely to be critical to both consistent year to year host infection and the evolution of virulence and resistance in the host and pathogen populations (Burdon et al. 1995;

Thrall and Burdon 1997). The spatial scale at which host plants exist relative to sources of inoculum, density of other plants, and conducive microhabitats is likely to determine whether plants become infected (Real and McElhany 1996) and to have direct impacts on plant fitness (Chilvers and Brittain 1972; Mitchell et al. 2002). In an agricultural system, Brunet and Mundt (2000) showed how the spatial pattern of mixed vs. pure stands of wheat influenced the relationship between *Puccinia striiformis* infection and seed production in wheat. Spatial patterns of susceptible hosts would likely be even more critical to disease dynamics in a natural system where spatial proximity of hosts to inoculum and plant diversity are highly variable. However, few empirical studies on host spatial pattern and disease dynamics in natural systems have been done (Thrall and Burdon 2003).

For plant pathogens, patterns of disease in space have

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been used to explain aspects of transmission or spread of the disease (Campbell and Madden 1990). It is often difficult to distinguish what determines the extent of the spatial distribution of plant disease, whether it results from physical constraints of pathogen dispersal (Roche et al. 1995; Jacobi et al. 1993), a property of the genetic variability of the host populations (Campbell and Madden 1990; Thrall and Burdon 2003), environmental variability, or some combination of factors. It can be difficult to distinguish physical constraints of dispersal from variation in genetic resistance because some plant pathogens induce disease only in specific combinations of the host and pathogen genotypes rather than all members of the host population (Person 1967). The Uredinales, or rust fungi are an important order of plant pathogens on crops, especially cereals, and on forest and cultivated trees. The spatial dynamics of infection by these fungi is especially intriguing because many rusts are heteroecious, requiring two hosts to complete their life cycle. In heteroecious rusts, basidiospores and aeciospores are dispersed from one host to infect the other. Heteroecism in rusts is assumed to be advantageous in promoting fungal survival under certain environmental conditions, in spite of the added risk of having to infect different hosts twice each season (Savile 1976), because of its persistence as a life history strategy.

Smeltzer and French (1981) described aeciospore dispersal gradients for *Cronartium comptoniae* (Arthur), rust on pine and sweetfern. They showed that aeciospore dispersal from the pine host was limited to 30 m; beyond this distance no infection was found on the sweetfern telial host *Comptonia peregrina* (L. Coult.). In pine-oak rust caused by *Cronartium quercuum* (Berk.) f.sp. *fusiforme*, Schmidt et al. (1982) predicted that although aeciospore counts decreased by 87% over a distance of 3–152 m, aeciospores were still available to generate infection 400 m from a heavily infected pine stand. These studies suggest that most infections by aeciospores from isolated sources occur over distances of tens of meters, but that small numbers of spores can travel greater distances.

In natural systems there is often more than one source of inoculum that may contribute to disease levels on a host. Typically primary or dominant inoculum sources are defined based on proximity to the host plant, but their influence on disease severity may be lessened because of more distal or secondary inoculum sources. In several wind-dispersed pathogens, including *Puccinia polysora* (corn rust) and *Phytophthora infestans* (potato late blight), Gregory (1968) showed that local spore sources are of greatest importance, but that secondary sources located at greater distances can contribute significantly to disease intensity and decrease the slope of the infection gradient. The impact of secondary sources can be significant enough in some instances to overcome the effect of local, or primary sources of inoculum (Gottwald et al. 1989). The importance of secondary inoculum sources is dependent on their spatial distribution, frequency, and intensity. *Puccinia andropogonis* (Schwein) Arthur, on its two hosts, *Comandra umbellata* (L. Nutt.), and *Andropogon gerardii* (Vitman), is an ideal natural pathosystem for investigating the importance of proximity between hosts in rust heteroecism because it has been largely unaffected by human activities such as resistance breeding

or disease management. *Puccinia andropogonis* is a macrocyclic, heteroecious rust commonly found in native tallgrass prairie systems in North America. It was once thought to have several telial host species, and more than 40 diverse alternate aecial hosts (see Jackson 1931; Arthur 1934; and Cummins 1953). However, Szabo and Roelfs (2002), using sequence analysis of the nuclear ribosomal ITS region, found the rust that cycles between comandra and big bluestem to be a distinct and separate species from forms cycling between other aecial hosts and big bluestem, or to little bluestem, *Schizachyrium scoparis* (Michx.). Therefore, complications due to multiple rust infections or misidentification of inoculum sources can be avoided by choosing study sites devoid of other hosts.

Comandra umbellata, commonly known as comandra, bastard toadflax, or false toadflax, belongs to the predominantly tropical sandalwood family Santalaceae. Comandra is a native perennial, dicot herb found throughout the US and Canada, and likely in every county in Minnesota (Bergdahl and French 1973). Comandra is the host of many pathogens, including the economically important comandra–pine blister rust *Cronartium comandrae* Pk. (Woods et al. 2000). Comandra occurs in characteristically dry, open to partially open sites (Piehl 1965), and is often associated with oak savannas dominated by black oak, bur oak, or northern pin oak (*Quercus velutina*, *Quercus macrocarpa*, and *Quercus ellipsoidalis* respectively; Curtis 1959). Comandra forms clones, which vary in size among years, ranging from a few to hundreds of stems that arise at intervals from horizontal underground rhizomes. It is thought to be common in ecosystems that rely on fire, possibly because fire functions to open the overstory (Piehl 1965).

Big bluestem (*A. gerardii*) is a native perennial, warm season grass that occurs in North America from the Rocky Mountains to the Atlantic Ocean. Along with indiagrass (*Sorghastrum nutans* (L.) Nash), and switchgrass (*Panicum virgatum* L.), big bluestem is an important part of the original fire-maintained tallgrass prairie ecosystem (Madson 1995), now found only as remnants. It is recognized as valuable forage for wildlife and domestic animals in the Great Plains (Pitman 2000). It is also commonly used for erosion control along roadsides and elsewhere partly because of its extensive root system (Madson 1995).

This study evaluates how distance between comandra and big bluestem influences rust severity on the telial host (big bluestem). The first objective of this study was to determine the importance of local sources of aeciospore inoculum on rust severity of big bluestem, and whether rust severity varied among years. This was done by evaluating the relationship between the total number of aecia on comandra (aecial density) within specific distance intervals from individual big bluestem plants and disease severity on those big bluestem plants from 1998 to 2001. The second objective was to use spatial autocorrelation analyses to measure disease aggregation on both plant hosts within each year of the study. A third objective was to establish whether the genetic background of big bluestem plants within the study site influenced disease severity using a common garden planted with big bluestem plants representative of the natural study site. Finally, a scheduled burn in the spring of 2000 in one of the two experimental fields allowed comparisons of host–

pathogen dynamics between fire-disturbed and nondisturbed fields.

Methods

Study site and plot establishment

Cedar Creek Natural History Area (CCNHA), a National Science Foundation Long Term Ecological Research (LTER) site (<http://www.cedarcreek.umn.edu>), rests on a glacial sandplain, and is composed of a mixture of prairie remnants, abandoned agricultural fields, oak savannas, hardwood and pine forests, and cedar swamps. CCNHA is located in east-central Minnesota (45°35'N, 93°10'W), approximately 50 km north of Minneapolis/St. Paul.

Big bluestem plots ($n = 66$ in 1998 and increased to $n = 80$ in 1999) were established in two separate fields 1.6 km apart (Fig. 1). The two fields were selected primarily based on the occurrence of comandra and big bluestem, but also for the lack of alternate hosts of other rusts that may also infect big bluestem (Arthur 1934). In each field, all comandra clones were identified and the nearest big bluestem plant or plants to each comandra clone were chosen as plots. Thus, each comandra clone was associated with one to at most three big bluestem plots. Of the 80 total plots, 18 plots were located in an oak savanna (Field 1), occupying an area of approximately 300 m × 300 m. This field is dominated by bur oak (*Q. macrocarpa*) with some northern pin oak (*Q. ellipsoidalis*), and is burned in four-year cycles. The trees in this field are relatively uniformly distributed, giving all big bluestem plots some shade protection during the day (Fig. 2). The remaining 62 plots were in Field 2. Plots within Field 2 tended to cluster into groups that varied in their microhabitat, including exposure to sunlight and distances between neighboring plots. Based on these differences, plots were placed into 8 groups to address differences in disease severity that could be attributed to plot location (Fig. 2). Field 2 is generally open with patches of bur oak (*Q. macrocarpa*), hazelnut (*Corylus americana*), smooth sumac (*Rhus glabra*) and green ash (*Fraxinus pennsylvanica*) around its borders and measures approximately 300 m × 275 m. All field dimensions and plot locations were mapped using a standard survey station by marking the center of each big bluestem plant and the outlines of each comandra clone. Distances between plants were tabulated using spatial analysis software by Arcview Inc. (ESRI 1998).

Rust scoring

Comandra rust was scored on 18 June 1998, 4 June 1999, 5 June 2000, and 6 June 2001, on every comandra clone by counting all aecia on every comandra leaf. However, when infection levels were very high, aecia would occasionally merge together and individual aecia could not be counted. Therefore, on heavily infected comandra leaves, a score of 10 aecia was assigned as an estimated maximum number of aecia a comandra leaf could bear. Big bluestem plants were scored for rust severity by choosing leaves randomly without regard to leaf age or position, and estimating percent leaf area infected by comparing the infected leaves to a standardized disease assessment scale (James 1971). Mean rust severity was obtained by scoring 30 randomly chosen leaves for each big bluestem plant in the study. Size, shape

and color of uredinial pustules were used to differentiate *P. andropogonis* from other diseases and leaf damage. Big bluestem was scored in the late summer, on 26 August 1998, 1 September 1999, 5 September 2000, and 31 August 2001. Scoring of rust severity was done by the same individual throughout the study.

Spatial analysis

Spatial autocorrelation analyses were performed for both mean rust severity on big bluestem plants and total number of aecia per comandra clone for each year of the study in Field 2 only. Moran's I (ROOKCASE version 0.9.5a; Sawada 1999) was calculated for both variables (rust on comandra and big bluestem) in each year of the study using the irregular lattice data format. In this study, Moran's I was calculated with a lag distance of 5 m, 10 m, and then by 10 m increments up to 90 m. Lag distance increments were chosen, in part, to be inclusive of the average distance between big bluestem plots (7 m) and the average distance between comandra clones (22 m). Lag distance is the defined distance at which pairs of data points are measured, and is used to quantify the correlation between values separated by that distance. Significant positive or negative spatial autocorrelations at a particular lag distance indicate the data are spatially dependent at that scale. Negative values indicate regularity and positive values indicate aggregation (Jumars et al. 1977; Cliff and Ord 1981; Legendre and Fortin 1989; Real and McElhany 1996). Significance testing of the Moran's I is done with a standard normal statistic (Z) by subtracting the expected value of Moran's I (assuming no aggregation) from the calculated value, and dividing by the standard deviation of the Moran's I (Campbell and Madden 1990).

Interaction between aecial density within specific distance intervals and rust severity on big bluestem

Aecial density was defined as the total number of aecia on comandra within specific distance intervals from individual big bluestem plants. Mean rust severity on big bluestem was regressed on aecial densities within eight distance intervals (≤ 1 m, ≤ 5 m, ≤ 10 m, ≤ 15 m, ≤ 20 m, ≤ 30 m, ≤ 40 m, and >40 m from big bluestem plants). The distance interval of >40 m was limited to a maximum of eight total comandra clones. The average distance between comandra clones and big bluestem plants of the over 40 m distance interval was 124 m in Field 1 and 66 m in Field 2. Aecial density within each distance interval was assessed as either exclusive of aecial density in other distance intervals, or as inclusive of all aecia present at shorter distances from the target plant. The power law model was used to describe mean rust severity on big bluestem as a function of aecial density within each distance interval separately (Gregory 1968; Fitt and McCartney 1986)

$$y = ax^b; \quad \text{linear form } \ln(y) = \ln(a) + b \ln(x)$$

where y is mean rust severity on big bluestem, a is a constant, b is the slope of the disease gradient, and x is aecial density within each distance interval. The linear form of the equation was used to fit a simple linear regression model.

Estimation of genetic variation within the study site

A common garden plot was established to estimate the genetic variability in resistance to *P. andropogonis* among big

bluestem plants. Seeds from 25 big bluestem plants were collected from Field 2. These plants were located at 20 m intervals along two transects that ran diagonally across the field. Additional seeds were collected from 15 randomly selected study plots in both fields. All seeds were collected on 4 February 2000 as the culms were above snow cover. Seeds were germinated in a greenhouse in individual pots, and 100 resulting plants were planted 1 m apart in a 10 m × 10 m garden 200 m northwest of Field 2 (Fig. 1). Plants in the garden plot resulted from 30 different parent plants, providing a potential for comparison of disease severities among siblings. The number of siblings per plant in the common garden ranged from 0 to 13. The same plants were scored for rust severity for all three years of the common garden study.

Garden plants were scored for rust severity using the same protocol as for field plots, and were assessed one day after field plots by the same individual. It was assumed that the virulence structure of the pathogen population does not vary significantly from year-to-year. The coefficient of determination (r^2), based on regression of individual plant rust severity in 2001 on rust severity in 2002, was used to estimate the degree to which genetic resistance and susceptibility affect rust severity. If genetic resistance plays a significant role in disease severity found on big bluestem, disease severity for individual plants should have a consistent ranking between years, giving a high r^2 value. Because the nearest big bluestem plants or comandra clones were >100 m from the common garden, it was assumed that plants have an equal chance of infection by spores from the same source, and exist under similar environmental conditions. In addition, rust severity among sibling plants should be consistent between years if there is a strong genetic component to disease resistance. This was investigated by using a one-way analysis of variance (ANOVA) of rust severity among sibling plants between years 2001 and 2002.

Data analysis

ANOVA (Montgomery 1991), unweighted ordinary least squares regression (Montgomery and Peck 1992), and Pearson's correlation coefficients (McClave and Sincich 2000), were calculated in this study.² Comparisons of means were done using Tukey's *t* test for multiple comparisons (Montgomery 1991). For all statistical tests, assumptions of equal variance were examined. Assumptions for equal variances for ANOVA tests were verified by Bartlett's test for equal variances (Snedecor and Cochran 1980). If the assumption was not met, the data were transformed by the natural logarithm and the assumption of equal variances was again evaluated. If assumptions were still not met, the nonparametric Kruskal–Wallis one-way ANOVA was performed, and the comparisons of mean ranks performed with $\alpha = 0.05$ (Montgomery 1991). Data from these tests were ranked and then a parametric procedure for computing a one-way ANOVA was used to generate *F* and *P* values (Conover and Iman 1981). In every case, the interpretation of the results was consistent with the χ^2 test performed on the rank-transformed data (Montgomery 1991), but only the *F* and *P* values are reported. For all regression analyses, residual plots were inspected for constant variance and linearity. As for

the ANOVA, if the assumptions were not met, data were natural log-transformed and reinspected. If the assumptions were still not met, the results were not considered and not reported. All analyses were run using Statistix for Windows software (Analytical Software 1998).

Results

Rust on comandra

There were significant differences in the mean number of aecia per comandra clone ($F_{[3,195]} = 8.41$, $p = 0.0001$, Kruskal–Wallis one-way ANOVA) among years (Fig. 3a). Differences in the number of aecia per comandra clone correspond to the significant differences among years in the number of comandra stems per comandra clone ($F_{[3,195]} = 14.94$, $p = 0.0001$; Kruskal–Wallis one-way ANOVA, Fig. 3b). In both 1999 and 2001, there were greater numbers of comandra clones with high numbers of aecia than in 1998 and 2000 (Fig. 4). Although the “U” shape of the figure may be an artifact of binning the data, the differences among years is noticeable. However, rust severity (as the mean number of aecia per comandra stem) did not differ significantly among years ($F_{[3,195]} = 0.37$, $p = 0.78$, ANOVA), although the variation among plants within years was high (Fig. 3c). These data demonstrate that aeciospore inoculum intensity can vary among years because of the changing size of comandra clones, even though disease severity on comandra may not fluctuate.

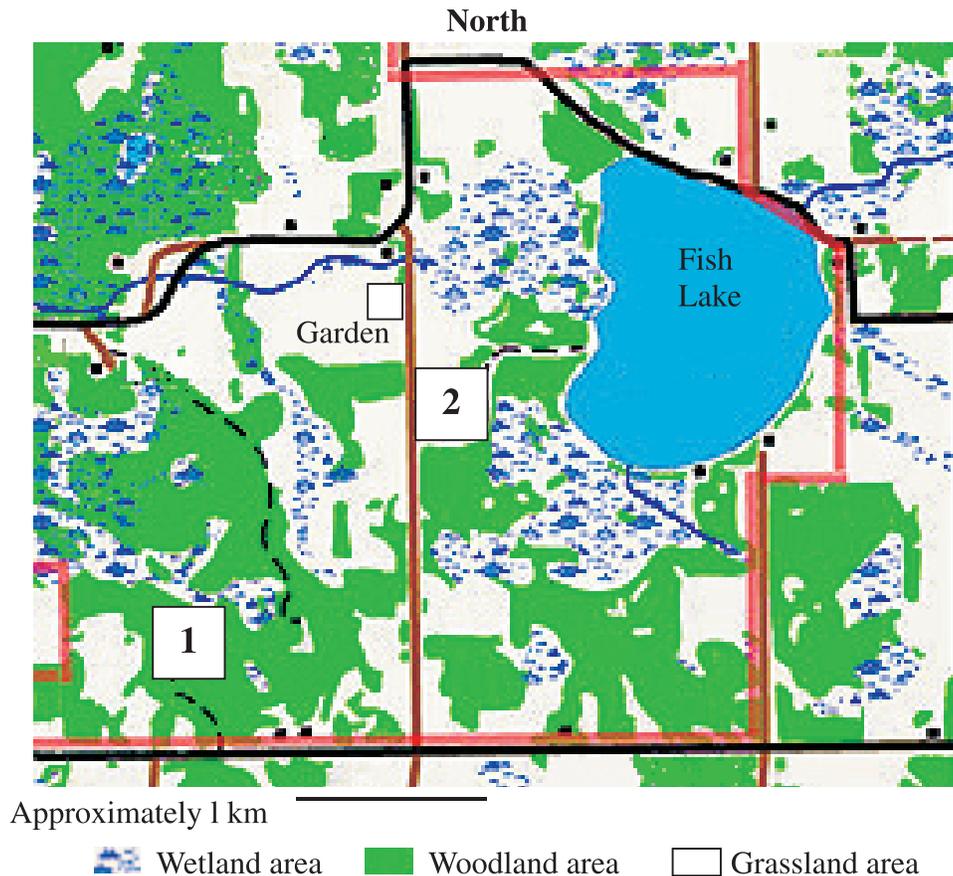
There were distinct differences among years within a field in the mean number of aecia per comandra clone, number of comandra stems per clone, and rust severity on comandra. In 2000, Field 1 was burned. In the year of the fire, Field 1 had significantly lower mean numbers of aecia per comandra clone ($F_{[3,44]} = 6.67$, $p = 0.001$; Fig. 3d), significantly fewer numbers of comandra stems per clone ($F_{[3,44]} = 12.5$, $p = 0.00001$; Fig. 3e), and significantly lower rust severity ($F_{[3,44]} = 4.1$, $p = 0.01$; Fig. 3f) than in most other years of the study. Significant differences among years in Field 2 occurred in the number of stems per comandra clone ($F_{[3,147]} = 12.37$, $p = 0.00001$; Fig. 3e), and in mean aecia per comandra clone ($F_{[3,147]} = 4.89$, $p = 0.003$; Fig. 3d). In Field 2, differences in mean numbers of aecia per comandra clone correspond to differences in the average number of comandra stems per clone among years. Annual fluctuations occurred in both fields even though only Field 1 was burned, suggesting variation in environmental conditions affecting comandra growth can significantly influence inoculum intensity among years. Furthermore, there were significant differences ($p < 0.05$) in the mean number of aecia per comandra clone among the geographically distinct groups in Field 2 (Fig. 2). Six out of eight groups in Field 2 had the highest average number of aecia per clone in 2001 (Table 1).

Rust on big bluestem

There were significant differences in the mean rust severity on big bluestem plants among the four years of the study ($F_{[3,297]} = 2.73$, $p = 0.043$; Kruskal–Wallis one-way ANOVA). Mean rust severity was significantly higher in 1998

²The raw data, in the form of a Microsoft Excel worksheet, can be found at <http://www.cdll.umn.edu/personnel/barnes.html>.

Fig. 1. Map of *Andropogon gerardii* and *Comandra umbellata* field locations established in 1998 at Cedar Creek Natural History Area. Field 1 (1) and Field 2 (2) are shown as squares and are approximately 1.6 km apart. One hundred *A. gerardii* were planted in a common garden plot located 200 m northwest of Field 2 (Garden) on 7 July 2000.



(5.50%) than in 1999 (3.38%), but not different from years 2000 (3.93%) and 2001 (4.43%). Variation in mean rust severity among plants varied among years (Bartlett's test of equal variances, $\chi^2 = 27.87$, $p = 0.0001$), and was greatest when mean rust severity was highest (1998 and 2001).

There were no significant differences in mean rust severity on big bluestem plants between Fields 1 and 2 in any year (Fig. 5). Variation in mean rust severity among plants was similar for the two fields, with the greatest variation occurring when mean rust severity was high. This suggests that yearly differences in rust severity on big bluestem may be affected more by environmental factors that influence both fields equally than by microclimatic or other differences that affect the fields individually.

Big bluestem groups varied in their relative disease severity rankings among years (Table 1). However, groups three and six had generally higher mean rust severities among years. These two groups are approximately 100 m apart, located in open areas away from bordering trees, and have plants that are comparatively tightly clustered compared with some other groups (see Fig. 2). Group 4, which is also in an open area of the field, had lower disease levels each year of the study compared to groups 3 and 6. The difference in disease levels was probably due to the greater average distance from comandra, and lower number of aecia on the nearest comandra clone (Table 1). Group 2 plants, which are as tightly clustered as plants in groups three and

six, also had lower average disease severity. However, plants in group 2 are predominantly under canopy cover, suggesting neighboring vegetation may reduce disease severity by limiting spore dispersal and (or) liberation, reducing outside inoculum levels by trapping spores on leaves and other plant tissues, or by changing the microenvironment.

Spatial autocorrelation

Rust was aggregated on both comandra clones and on big bluestem plots. Significant positive spatial autocorrelation (I) was found for both mean aecia per comandra clone and mean rust severity of big bluestem plots in Field 2. Values of I significantly different from zero ($p < 0.05$) were found at lag distances beginning at 20 m for comandra in 1998 and 2001, and 30 m in 1999 (Table 2). However, the nonsignificant values of I at lower lag distances may result from the spatial distribution of comandra clones within the study site because there are few comandra clones present at closer distances. No significant values of I were found for mean aecia per comandra clone at any distance in 2000, and values of I were not significant at distances >60 m in 1998 and 1999. Significant positive values of I were found up to 90 m in 2001 (Table 2). This likely reflects the significant increase in the mean aecia per comandra clone that year and consequently greater "source strength" (Fig. 3d). Generally, aggregation was highest at distances roughly equivalent to the size of the geographic groups (about 40 m), suggesting that

Fig. 2. Maps of *Andropogon gerardii* – *Comandra umbellata* plots in two fields at Cedar Creek Natural History Area. Each solid circle represents a single *A. gerardii* plot, and open diamonds represent the outline of *C. umbellata* clones. Field 1 measures approximately 300 m × 300 m, while Field 2 is 300 m × 275 m. The large dashed ovals in Field 2 show the subdivision of plots into geographically similar groups. The block outlines represent forested areas. Field 1 is an oak savanna where most plots have an overstory, in contrast to Field 2, where most plots are in the open, with the exception of group 1 (overstory like Field 1), and the southern part of group 2.

high numbers of aecia were confined to relatively small areas of the field.

Mean rust severity on big bluestem was positively spatially autocorrelated at 5 m in 1998, 2000 and 2001 (Table 2). Aggregation below 5 m was not detected due to insufficient sample size (Legendre and Fortin 1989) and is a function of the experimental design that limited the number of neighboring big bluestem plots at distances less than 5 m. The highest degree of autocorrelation for rust severity on big bluestem at distances less than 10 m suggests that the observed patch size (an area defined by the number of adjacent plants with significant autocorrelation values for rust severity) is one that would often include only one or two adjacent big bluestem plants (Jumars et al. 1977).

Significant values of I for rust on big bluestem, although small, were found up to a lag distance of 90 m in 2001. This likely reflects the significant increase in the average total aecia per comandra clone and the significant aggregation of rust on comandra at greater distances that year. The value of I varied substantially among years for both rust severity on big bluestem and mean number of aecia per comandra clone, which indicates rust infection on either host was not consistently high in one particular location among years of the study.

Interaction between aecial density within specific distance intervals and rust severity on big bluestem

Using the power law model, there was a significant positive relationship ($p < 0.01$) between mean rust severity on big bluestem and aecial density on comandra at the first seven inclusive distance intervals in 2000 and 2001 (Fig. 6a). In 1998 and 1999, the relationship between mean rust severity on big bluestem and aecial density on comandra was significant beginning at the 5 m distance interval ($p < 0.001$). No significant relationship was found in any year at the >40 m distance interval, or in 1999 at distances >20 m. The slope and coefficient of determination (r^2) generally decreased with increasing distance (Fig. 6a and 6b), suggesting that the strength of the relationship between comandra aecial densities and big bluestem rust severities decreases with distance. The significantly higher slope and r^2 values in 2001 corresponded with the significantly higher aecia numbers per comandra clone and the higher number of comandra stems per clone seen that year (Fig. 3), suggesting that higher local aecial densities strengthen the relationship between aecial numbers and rust severity on the grass host. Regression analyses detected no significant relationship between the number of aecia and rust severity on big bluestem at distances over 40 m, suggesting that comandra clones within 40 m predominate as inoculum sources. Absence of significant relationships at distances <1 m in 1998 and 1999 (Fig. 6a) may be an artifact of the distance intervals because most comandra clones are >1 m in diameter.

The power law model was also fit to the data by regressing the Ln of mean rust severity on big bluestem on the Ln of aecial density on comandra clones at each distance interval separately. There was a significant positive relationship of mean rust severity on big bluestem to aecial density on comandra at <1 m in 2000 and 2001 ($p < 0.05$ and $p < 0.002$, respectively). Absence of significant relationships at distances <1 m in 1998 and 1999 (Fig. 6a) may be an artifact of the natural distribution of host plants as mentioned above. There was a significant positive relationship ($p < 0.002$) between mean rust severity on big bluestem and aecial density on comandra between 1 m and 5 m in all years of the study. However, no significant relationship between mean rust severity on big bluestem and aecial density on comandra was found at distances greater than 5 m in any year of the study.

In Field 2 there was a positive relationship between rust severity on big bluestem and aecial density on comandra at roughly the same distance intervals as was observed for the combined (fields combined) data, with greater distance intervals becoming significant in 1999 and 2001 (Fig. 6c). There was no significant relationship between rust severity on big bluestem and aecial density on comandra in Field 1 at any distance interval. Thus, the short-term, four-year burning schedule in Field 1 may weaken the relationship of mean rust severity on big bluestem to aecial density on comandra by reducing population densities of one or both hosts compared with Field 2. The equality of the overall rust severity on big bluestem between fields in 2000 (Fig. 5), suggests Field 1 was likely infected by distal sources of inoculum outside the field that year. The strength of the relationship between rust severity on big bluestem and aecial density on comandra was determined for both Field 1 and Field 2 by calculating Pearson's correlation coefficients for each distance interval. Differences between fields can be seen in the consistent positive correlation between rust severity on big bluestem and aecial density on comandra seen in Field 2, and a change from a positive to a negative correlation between rust severity on big bluestem and aecial density in Field 1 in 2000 and 2001 (Fig. 7). These results suggest that while local sources of inoculum are consistently correlated with rust severity in Field 2, the fire in Field 1 can negate that relationship and increase the importance of more distal sources of inoculum to maintain infection in that field.

Common garden

Andropogon gerardii plants in the common garden had significantly lower mean rust severities the first year 2000 (0.58), when plants were small, than in 2001 or 2002 ($F_{[2,296]} = 45.74$, $p < 0.0000$). Mean rust severities in 2001 (3.77), and 2002 (3.62) did not differ significantly ($p > 0.05$). Regression analyses were performed by regressing mean rust severity on big bluestem plants in 2001 to 2002 values to evaluate the relationship between rust severity on

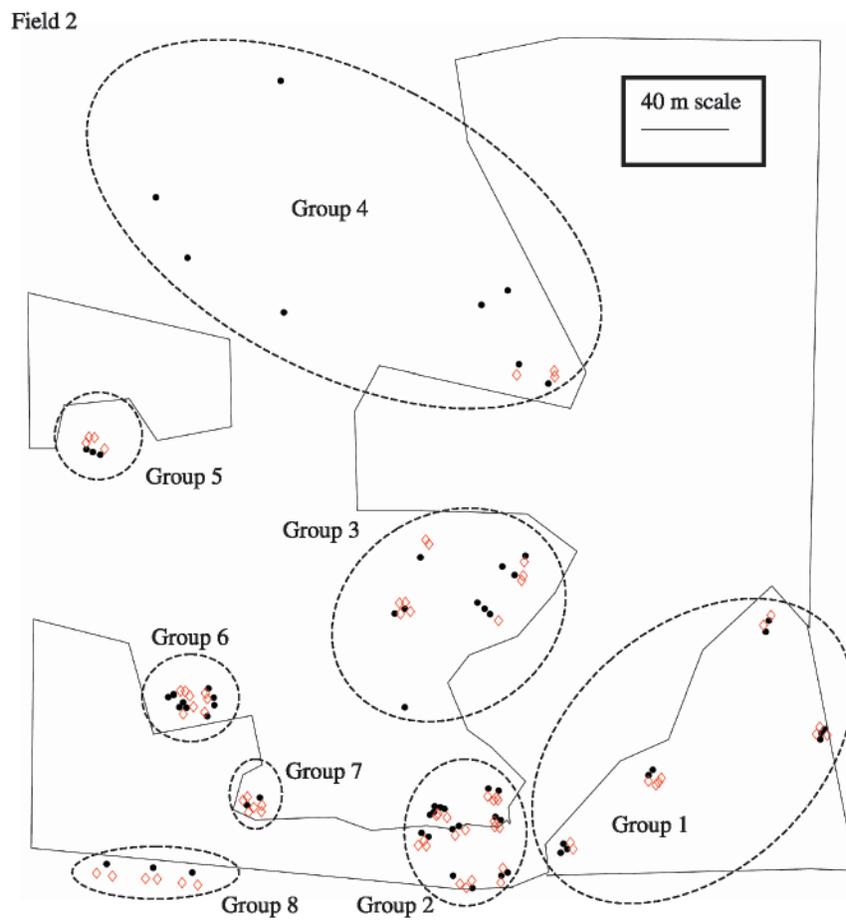
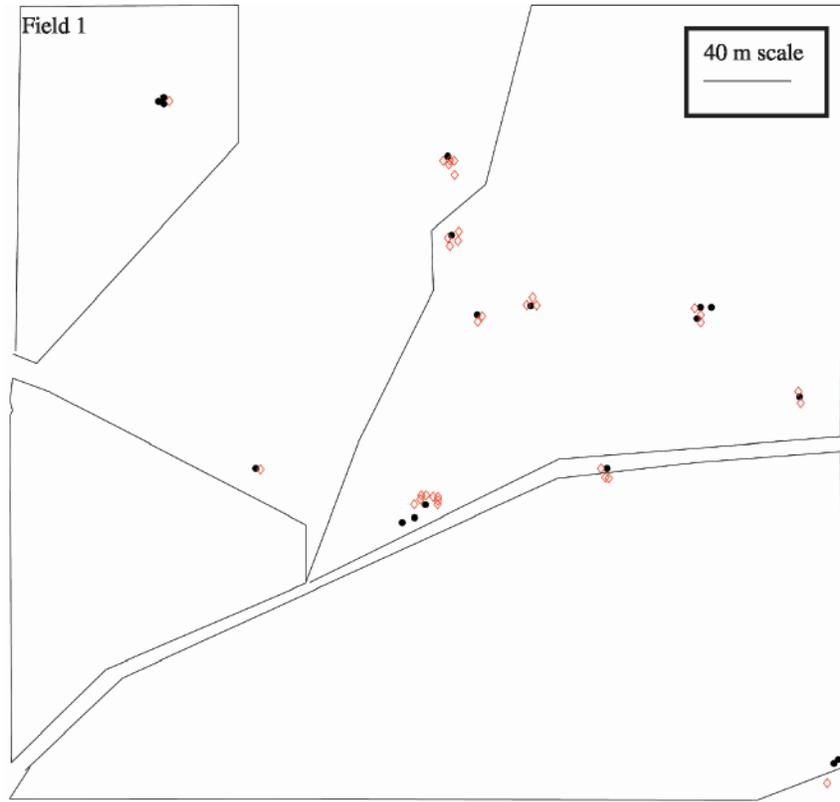


Fig. 3. Variation in mean aecia per comandra clone, mean numbers of stems in each comandra clone, and mean aecia per stem. Data from combined fields (a, b, and c) and fields shown separately (d, e, and f). Error bars indicate the standard errors of the means.

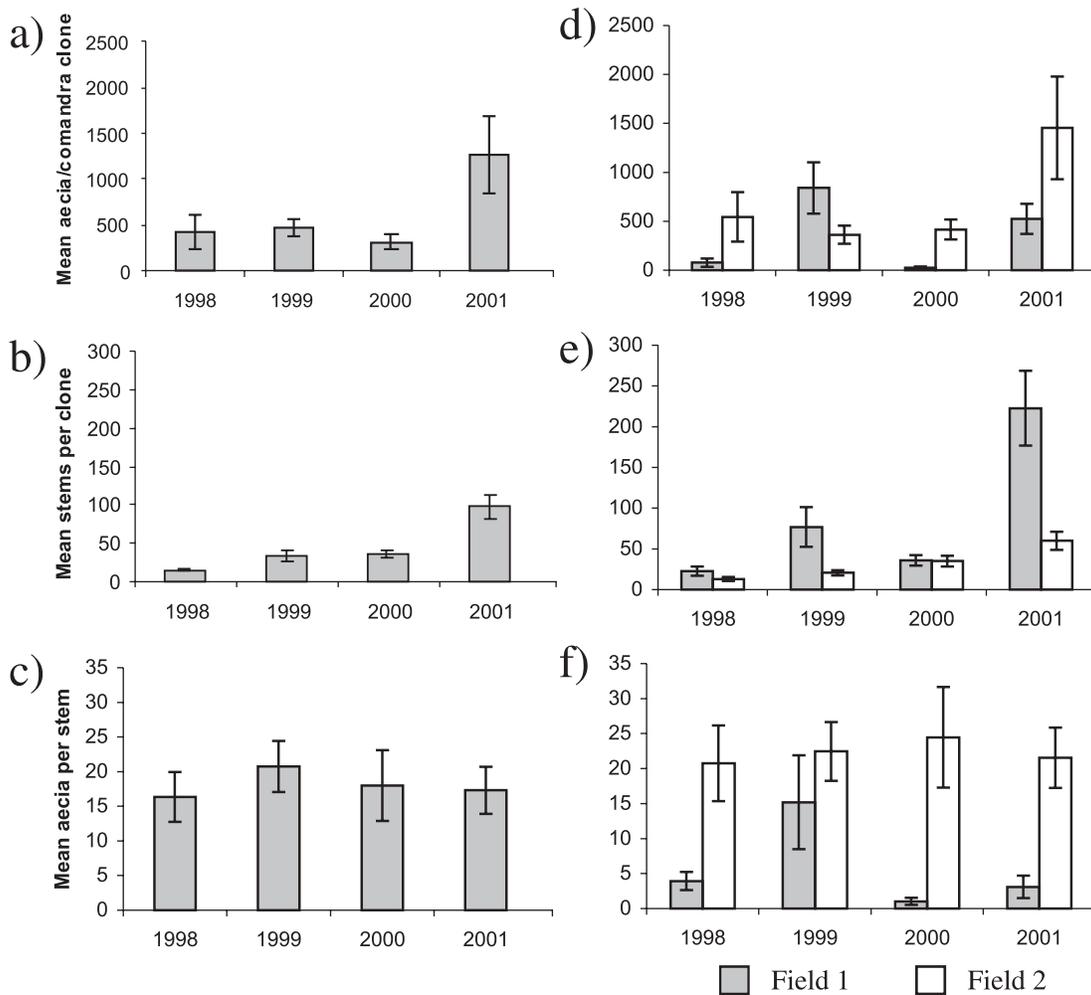


Fig. 4. Frequency distribution of the number of *Comandra umbellata* clones within categories of total aecia per clone. Total number of comandra clones was 49 in 1998, 56 in 1999, 54 in 2000, and 58 in 2001.

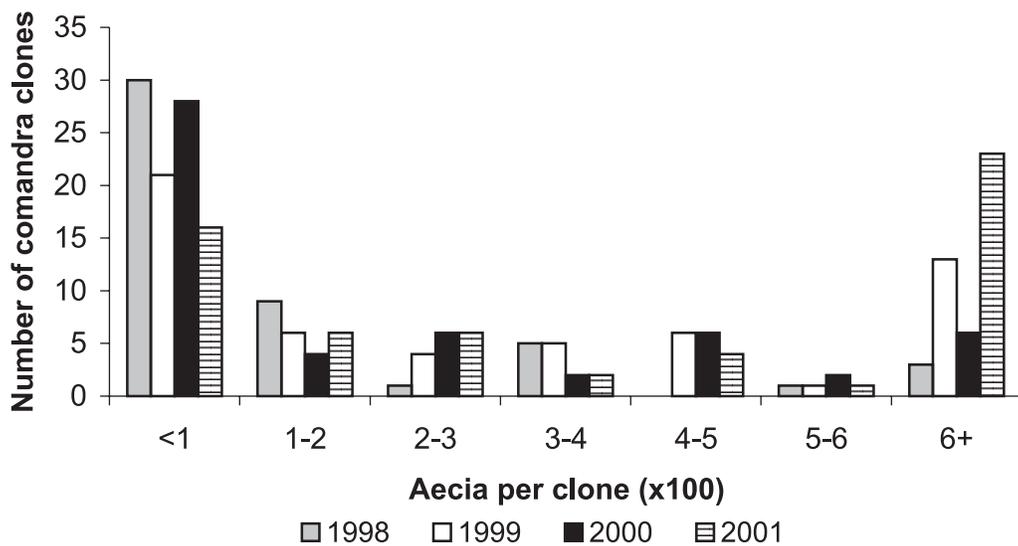


Table 1. Average number of aecia per *Comandra umbellata* clone (Cu) and mean rust severity for *Andropogon gerardii* (Ag) in each of the eight groups in Field 2 (GP1–8) during the four years of the study.

	1998		1999		2000		2001	
	Cu	Ag	Cu	Ag	Cu	Ag	Cu	Ag
GP6 (9)	176b	7.5a	372bc	5.3a	415b	7.3a	1028b	7.0a
GP3 (10)	413b	8.4a	1039a	5.2a	1116a	5.4ab	1965b	6.1ab
GP7 (2)	265b	4.6a	900ab	3.3a	707ab	3.8ab	1195b	5.4abc
GP8 (3)	3233a	6.6a	120bc	2.4a	15b	1.8b	6299a	7.0abc
GP2 (17)	50b	6.1a	217bc	3.1a	300b	3.9b	215b	4.0abc
GP1 (10)	82b	3.8a	78bc	2.6a	586ab	2.4b	185b	1.2c
GP4 (8)	30b	4.0a	8c	3.5a	8b	3.5b	121b	1.3bc
GP5 (3)	100b	3.0a	199bc	1.7a	170ab	2.1b	769b	1.7abc
Mean	544	5.50	366	3.39	415	3.8	1462	4.2

Note: Means within a column followed by different letters are statistically different ($p < 0.05$, Tukey HSD). Groups are ranked from the highest total rust severity on *A. gerardii* to the lowest by summing yearly averages and are identified in Fig. 2.

individual plants among years. The coefficient of determination of mean rust severity on individual plants between years was low ($r^2 = 0.26$, $b = 0.4 \pm 0.07$, $p = 0.0001$), suggesting that plants did not show consistent differences in resistance and susceptibility. Differences in the location of disease foci among years can be seen in the successive contour plots (Fig. 8). The shifting of the foci in successive years shows that disease foci were not associated with differences in resistance among plants. Consequently, the results of the common garden experiment suggest that a strong difference in resistance among big bluestem plants in the fields is not obscuring the distance–disease relationship.

The absence of consistent differences in susceptibility to *P. andropogonis* can also be seen when comparing rust severity on sibling big bluestem plants. ANOVA within each of the nine siblings groups (plants with ≥ 5 siblings) showed no greater similarity among siblings than among nonrelated plants ($p > 0.05$ for all comparisons). Additionally, each sibling group had individuals with significantly higher rust severities in different years ($p < 0.05$).

Discussion

It has been postulated that the spatial structure of host plants in a natural system will have a significant impact on disease persistence among years (Real and McElhany 1996; Thrall and Burdon 1997; Thrall and Burdon 2003), but few empirical studies have evaluated the effects of distance from multiple and randomly dispersed inoculum sources on disease severity on a naturally-occurring plant host. This work shows that the spatial structure of the alternate host population in the *P. andropogonis* rust pathogen system has a strong effect on aeciospore infection of big bluestem. Significant correlation between aecial density on comandra and rust severity on big bluestem was observed at a distance of 1–5 m between hosts when analyzed excluding other distances. However, local increases in aecia on the alternate host correlate directly with increases in rust severity on the telial host to about 40 m when increasing distances are inclusive. The significant relationship between the number of aecia on comandra clones and big bluestem infection was found in

each of the four years of the study, suggesting the effects of comandra infection on rust severity on big bluestem are consistent over time.

The significance of a distance of 40 m on the relationship between the number of aecia on comandra and rust severity on big bluestem may reflect both the physical limits of dispersal as a function of the dilution of spore concentration over distance, and the spatial distribution of comandra clones. Smeltzer and French (1981) showed that aeciospore dispersal for *Cronartium comptoniae* was limited to 30 m where beyond this distance no infection was found on sweetfern. Assuming similar dispersal dynamics, a corresponding distance limitation seems likely to affect the dynamics of aeciospore infection of big bluestem at CCNHA. In Field 2, comandra clones clustered at a scale of about 40 m and a significant relationship between the numbers of aecia on comandra with rust severity on big bluestem was found each year of the study. However, no clustering of comandra clones was found in Field 1, and no significant relationship was found between the numbers of aecia on comandra and rust severity on big bluestem plants in that field, even though all big bluestem plants were infected each year. These results suggest that clustering of infected comandra plants has the greatest impact on rust severity on big bluestem when the clusters and corresponding inoculum production become large enough to increase inoculum density to an apparent threshold level at which there is a significant relationship with rust severity on the telial host.

The growth dynamics of comandra had a significant effect on total inoculum and on *P. andropogonis* infection. Fluctuations in the number of comandra stems produced fluctuations in inoculum availability that corresponds directly to changes in rust severity on big bluestem. It is important to note that *C. umbellata* reaches its highest densities in the oak savannas typical of CCNHA (Curtis 1959) and after fire (Piehl 1965). Therefore, disease incidence on comandra may be higher and more persistent in these particular study fields because of the favorable environmental conditions for comandra growth, leading to more consistent infection of big bluestem in the same locations.

Host density has been shown to be significantly correlated

Fig. 5. Rust severity of *Puccinia andropogonis* on *Andropogon gerardii* in Fields 1 and 2 over the four years of the study. Error bars indicate standard errors of the means.

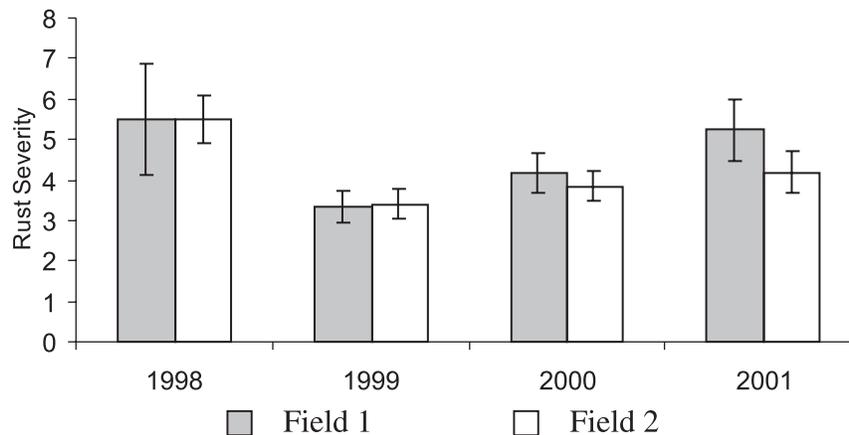


Table 2. Significant values of Moran's *I* for the average number of aecia per *Comandra umbellata* clone (Cu) and mean rust severity on *Andropogon gerardii* (Ag) at various lag distances during the four years of the study ($p < 0.05$).

Lag distance (m)	1998		1999		2000		2001	
	Cu	Ag	Cu	Ag	Cu	Ag	Cu	Ag
5		0.48				0.53		0.39
10						0.46		0.19
20	0.78					0.40	0.75	0.18
30	0.46	0.14	0.28			0.29	0.52	0.16
40	0.31		0.39			0.19	0.4	0.13
50	0.26		0.32			0.15	0.36	0.15
60	0.26		0.26			0.11	0.34	0.14
70							0.21	0.13
80							0.16	0.10
90							0.12	0.05

Note: The average distance between *C. umbellata* clones is 22 m, and the average distance between *A. gerardii* plots is 7 m.

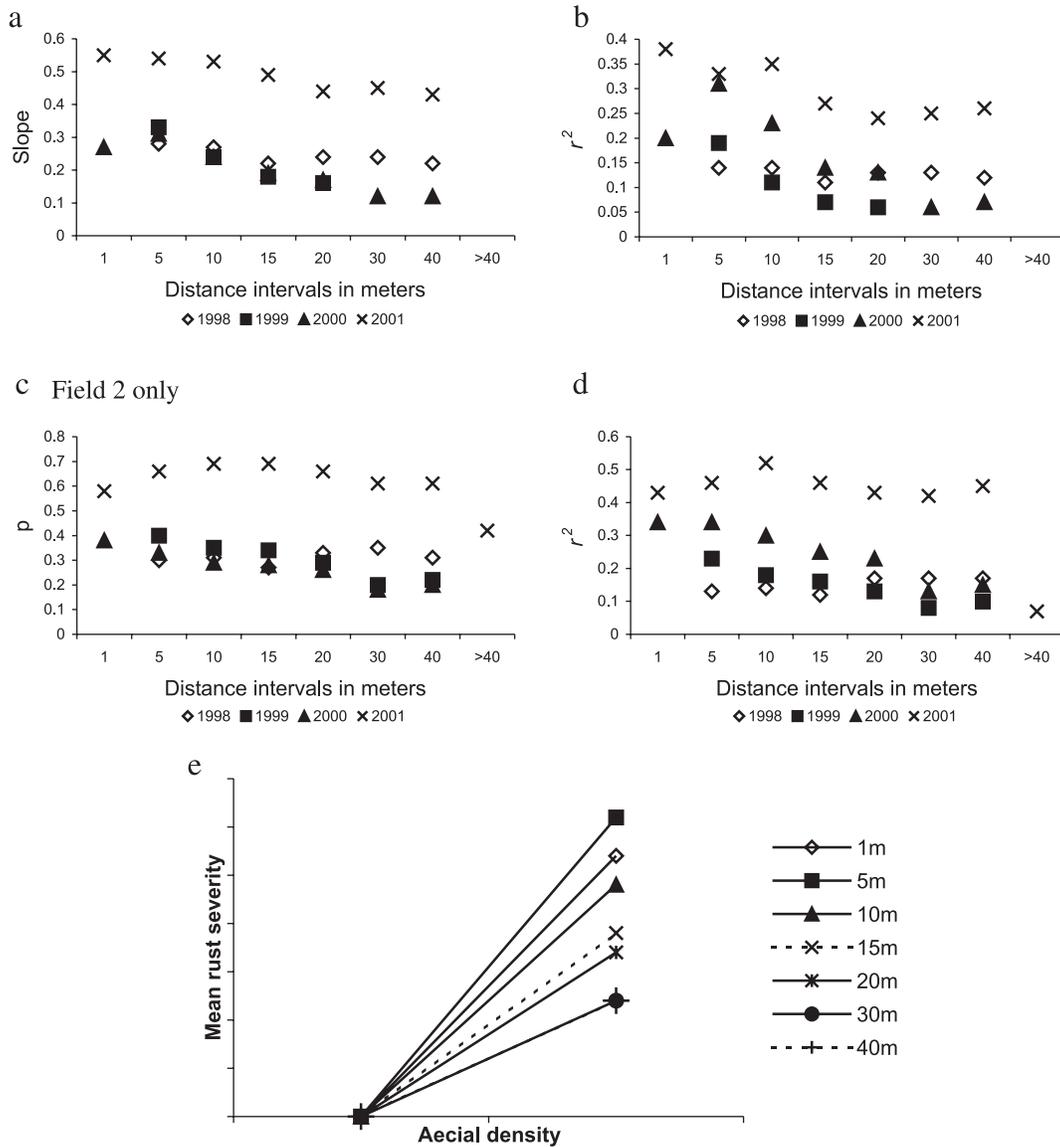
with disease severity in natural systems (Burdon and Chilvers 1982; Mitchell et al. 2002). Both comandra clones and big bluestem plants in groups of higher plant densities had on average higher numbers of aecia on comandra and rust severity on big bluestem. However, the current study also suggests that the position within the field, relative to tree canopy for example, can influence disease severity. Groups of comandra and big bluestem plants with approximately equal host densities, but located predominately under canopy cover, had lower rust intensities on both hosts, possibly because of the surrounding vegetation trapping and filtering out spore inoculum (Stakman and Harrar 1957), or because of changes in the local microclimate. These surrounding plants may also decrease wind velocity, thereby affecting spore liberation (Aylor et al. 1981; Sutton et al. 1984) and transport (Cammack 1958; Fitt and McCartney 1986; Jacobi et al. 1993), thus limiting local dispersal and deposition of all spore stages of the rust life cycle among neighboring plants.

Fire significantly changed the importance of proximal sources of aeciospores to infection of big bluestem most likely by reducing the number of teliospores in Field 1 in the spring of 2000. The transition from consistently positive to nonpositive correlations between aecial density (the num-

ber of aecia on comandra within a give distance interval) and mean rust severity on big bluestem suggests there was a switch from local (within field) inoculum to an outside inoculum source for two years following the fire. While the spring fire resulted in a significant decrease in the number of aecia on comandra in 2000, comandra growth was significantly enhanced in 2001. While there was not an increase in the mean number of aecia per comandra stem in 2001, the significant increase in comandra stems resulted in a significant increase in total inoculum (total aecia per comandra clone). The persistence of the rust in the years after a fire may be aided by the enhanced growth and vigor of the comandra host following the fire. Local sources of aeciospores could then become an important inoculum source to neighboring big bluestem plants very quickly. Inferring a similar effect from when Field 1 was burned in 1996 to the state of rust on comandra was first observed in 1998, it appears that while numbers of aecia per comandra clone decrease the year of a fire, they reach levels found in the undisturbed field (Field 2) within two to four years and become positively correlated to rust severity on big bluestem.

Rust severity on big bluestem was not different between fields in any year of the study even though fire significantly reduced the number aecia on comandra in Field 1 in 2000.

Fig. 6. Slopes (a) and coefficients of determination (b) resulting from regression analysis where the power law model was fit to the data by regressing mean rust severity on *Andropogon gerardii* to aecial density on *Comandra umbellata* clones at eight distance intervals from *A. gerardii* plots. Only significant unweighted least-squares regression analyses are shown ($p < 0.05$). Slopes (c) and coefficients of determination (d) resulting from the same regression analysis, but for Field 2 only. A representative example (e) of slopes associated with each distance interval. Intervals of 30 m and 40 m have the same slope.

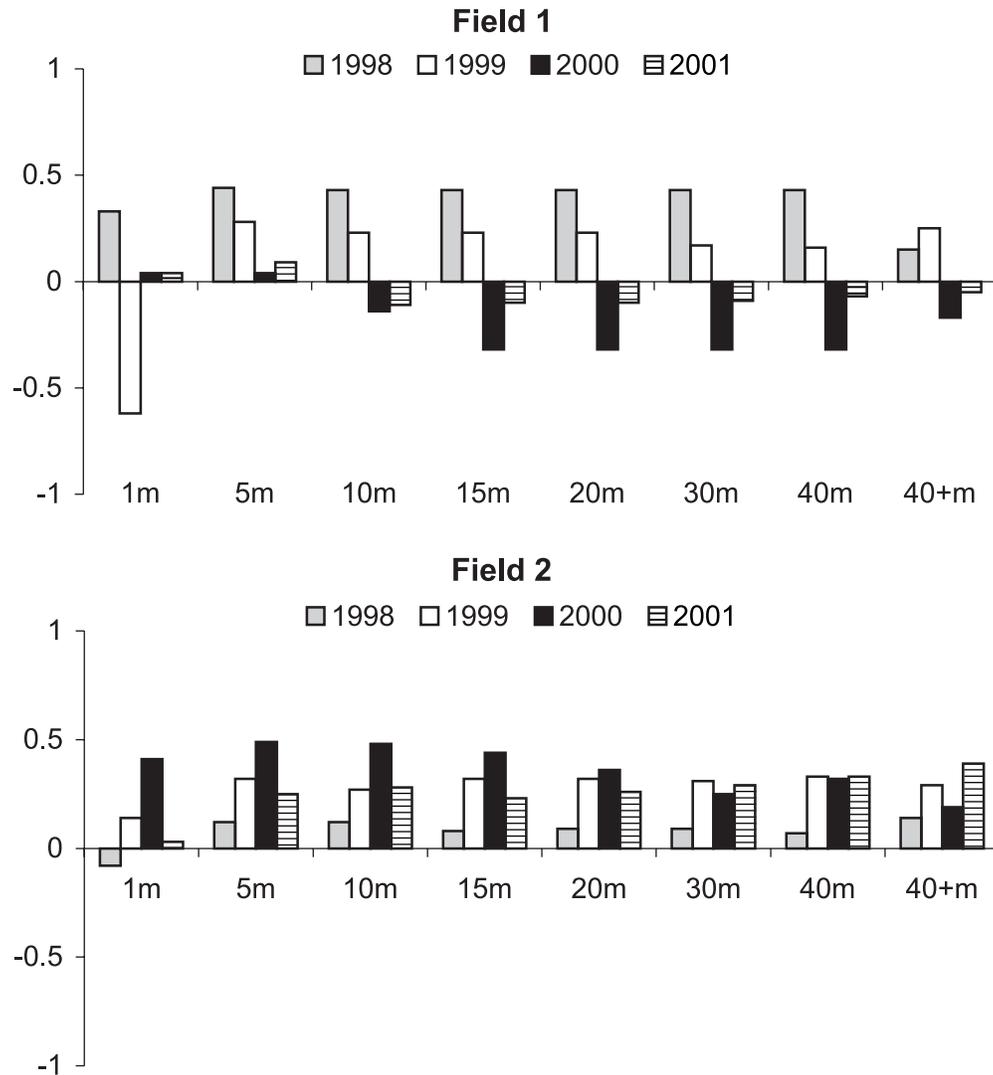


Big bluestem, like other prairie grasses, is adapted to fire (Daubenmire 1968; Pemble et al. 1981; Madson 1995; Whelan 1995). The spring fire in Field 1 had a positive effect on the growth of big bluestem the same year, and there was a visible difference between Fields 1 and 2 in big bluestem plant size (data not shown). Groth and Urs (1982) found that bean rust (*Uromyces phaseoli*) is more successful in colonizing younger leaves, where more and larger uredinia are produced than on older leaves. The flush of leaves of big bluestem after the fire might cause a similar increase in early rust buildup. Specifically, increased inoculum exposure may result due to increased leaf size and more rapid early growth. Early infection may also increase the possibility of additional uredinia cycles on those plants, although this was not measured. These effects could have offset the decrease

in aeciospore inoculum following the fire, explaining the lack of difference in rust severity in Fields 1 and 2 in 2000.

The results of the common garden experiment suggest that a strong difference in general resistance among big bluestem plants is not obscuring the distance–disease relationships found among field plots. The lack of consistency in disease intensity of individual plants in successive years suggests that plants are not showing strong differences in general resistance and susceptibility. It is important to note that the source of inoculum is a population and not single isolates. Because the nearest comandra clone is >100 m from the garden, the noncorrelated, year-to-year variation in the garden among plants most likely reflects the early arrival of a few aeciospores or urediniospores resulting in randomly located foci among years. This is consistent with the hypoth-

Fig. 7. Pearson correlations between aecial density (number of aecia on *Comandra umbellata* clones within each distance interval) and mean rust severity on *Andropogon gerardii* at eight distance intervals from *A. gerardii* plots. No correlations were significant in any year in Field 1 ($p > 0.05$). In Field 2, nonsignificant correlations were found in 1998, at intervals <1 m, or intervals >40 m in 2000. Otherwise, all correlations in Field 2 were significant ($p < 0.05$).

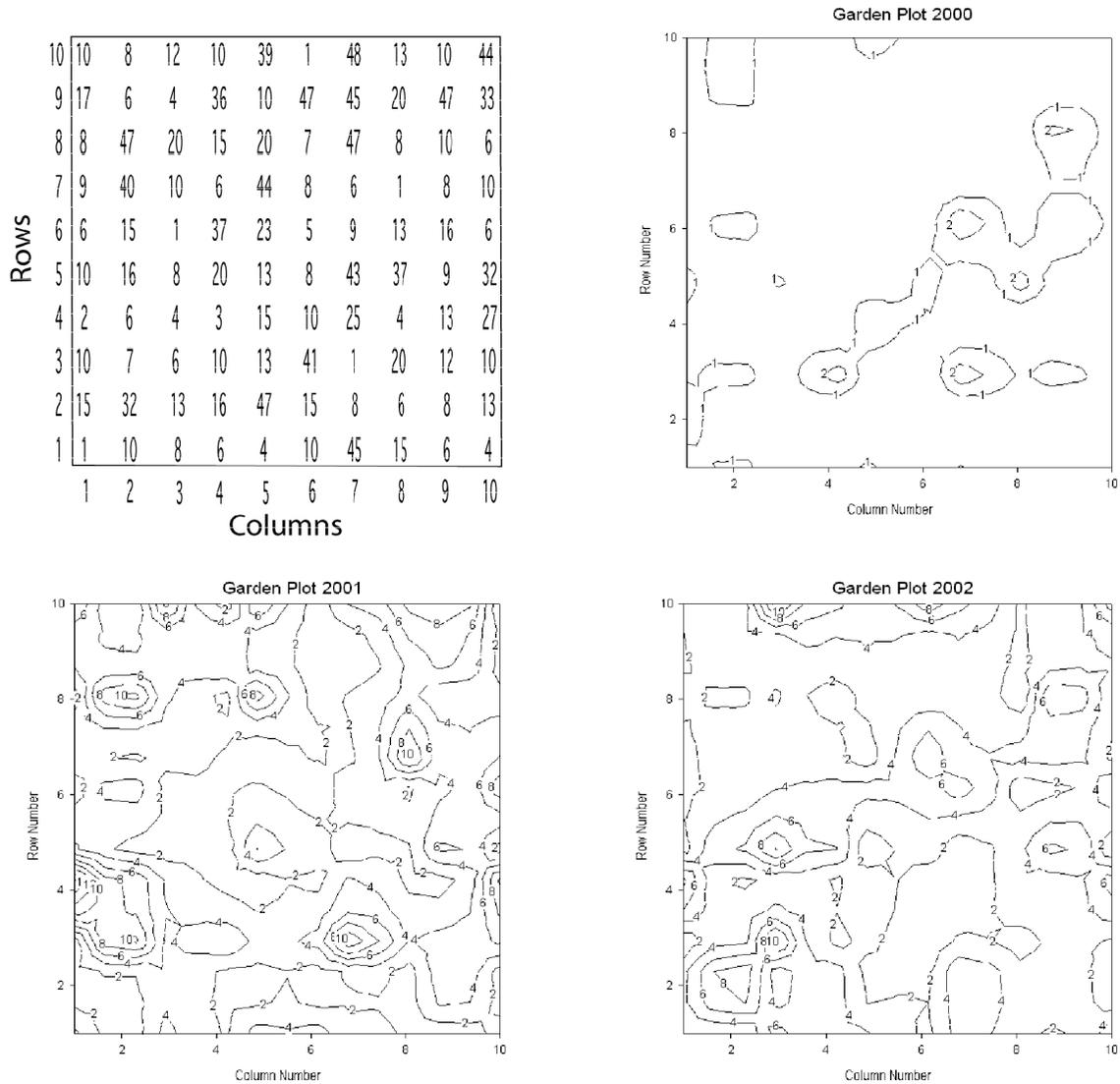


esis that there are not strong differences in resistance among big bluestem plants. Alternatively, variation in disease intensity on individual plants among years could result from infection by genetically distinct rust populations infecting the garden plants in different years, suggesting specific resistance and virulence within the plant and the fungus, respectively. To confirm this, controlled inoculations with pure isolates of the rust would need to be performed. However, the formation of genetically different rust and host populations is unlikely at CCNHA, since *P. andropogonis* primary inoculum is derived annually from sexual reproduction and big bluestem is an outcrossing host.

The absence of strong differences in resistance and susceptibility among big bluestem plants in this study supports theoretical expectations proposed by Thrall and Burdon (1997). They hypothesize that as spatial and temporal variability in the pathogen population declines and the time disease is absent from the system decreases, conditions are approached where race-specific resistance might confer little

benefit. At CCNHA, the prevalence of the rust is very stable: all big bluestem plants that were observed were infected every year. Under such conditions, selection tends to favor race nonspecific resistance (Thrall and Burdon 1997) rather than gene-for-gene interactions that produce frequent local pathogen extinctions (Burdon et al. 1996). The results of the common garden plot that there are no strong differences in resistance and susceptibility among big bluestem plants in this system are consistent with and support the theoretical model. The effect of the outcrossing population of big bluestem on rust population substructure is in contrast to the effect of tight inbreeding in wild flax (*Linum marginale*) found by Thrall and Burdon (2003) on the population substructure of flax rust (*Melampsora lini*). The tight inbreeding of wild flax resulted in a nonrandom spatial distribution of resistance among local plant populations. Subsequently, strongly adaptive local rust populations developed within the local flax populations as a consequence of the tradeoff between spore production and virulence (ability to infect

Fig. 8. Map of big bluestem plants and contour plots for 2000, 2001, and 2002 for the big bluestem planted garden. Numbers in the map represent the position along the transect of the parent plant from which seed was collected, and duplicate numbers indicate siblings. Values in the contour plots are of mean rust severity. Disease severity foci were not consistent over the three years.



multiple host genotypes). They found that virulence of a given pathogen population increased directly with the mean resistance of plant populations, suggesting that the evolutionary trajectories of both the flax and rust paralleled each other based on differences in the selective environment generated by the host. If the flax-rust system is analogous to the big bluestem-rust system, it would follow that an outcrossing big bluestem population and sexually recombining *P. andropogonis* populations are probably equally heterogeneous, supporting the theory that there is little population substructure of resistance or virulence in either big bluestem or *P. andropogonis* in this locality.

Plant pathogens have an impact on the structure and evolution of natural plant communities (Gilbert 2002; Mitchell et al. 2002). Mitchell et al. (2002) showed that as species diversity decreased, disease severity increased for many grassland plant species at CCNHA, and that certain plant species have more of an impact on disease levels than others. However, the specific influence of heteroecious rusts

on plant communities is unique in that rusts require two host plants to maintain their life cycle. Heteroecious rusts have been considered to reduce competition between plant hosts (Rice and Westoby 1982; Shattock and Preece 2000), and could therefore increase plant diversity by also reducing competitive effects of infected hosts on other species. However, for rusts to have an impact on the growth of either host, the distance between hosts must be within the range of spore dispersal. This study clearly demonstrates the significance of distance between hosts on rust severity of both hosts and the scale at which this is evident in a naturally-occurring plant community. Thus, while the spatial distribution of the hosts maintains the life cycle of the rust population, it may also sustain the coexistence of the hosts.

This empirical study demonstrates the importance of the spatial proximity of the alternate and telial hosts on rust severity in a natural prairie system. The remarkable consistency and significance of the power law model in describing rust severity on big bluestem relative to proxim-

ity of aecial density among years of the study underscores the importance of aeciospore infection in this host-pathogen system. Ultimately, the constraints of spore dispersal may have led to, or been a consequence of *P. andropogonis* becoming a species-complex infecting a range of aecial hosts. (see Arthur 1934; Cummins 1953; Szabo and Roelfs 2002). In the classic example of stem rust (*Puccinia graminis* f.sp. tritici), eradication of the alternate host has led to rust infection on wheat predominantly by asexual urediniospores (Roelfs 1982). In contrast, the close proximity of naturally-occurring plant hosts and consistency of infection in nonagricultural systems may increase the relative importance of sexually-derived aeciospores and perpetuate the coevolutionary dynamics between the hosts and the rust pathogen. Therefore, the genetic heterogeneity of *P. andropogonis*, generated through consistent sexual recombination is maintained because of the spatial structure of the hosts, and the spatial structure of the hosts is dependent on the adaptation of the hosts to particular environmental conditions, including fire.

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