MAPPING THE CHOKE PATHOGEN IN CULTIVATED ORCHARDGRASS FIELDS IN THE WILLAMETTE VALLEY

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Introduction

The choke disease pathogen (*Epichloë typhina*) is an endophytic fungus that was inadvertently introduced into cultivated orchardgrass fields in western Oregon. The fungus was first reported in 1996 in Oregon, but has quickly spread to ~90% of cultivated orchardgrass seed fields in the region, causing yield losses up to 65% in individual fields (Pfender and Alderman, 2006). The fungus develops intercellularly and maintains systemic endophytic growth in aerial vegetative host tissues. When the host plant enters its reproductive phase, branched hyphal masses (stromata) form externally on grass culms, and occasionally on vegetative tillers (Schardl, 1996). This affects the emergence of the inflorescence and, as a result, no seeds are produced on the affected tillers. Hence, the expression of *E. typhina* in host grasses is called "choke disease."

The choke pathogen must sexually outcross in order to produce ascospores (i.e. infective propagules of the fungus). A fly, hereafter referred to as the choke fly (*Botanophila lobata*), serves as a "pollinator" for the fungus (Bultman et al., 1998). Female choke flies visit the fungus to feed, and to lay solitary eggs on the stroma. During an oviposition behavior, the fly defecates previously consumed fungal spermatia, enabling cross fertilization of the fungus as it drags its abdomen along the stroma surface.

The present study was conducted to gain insights on the spatial distribution of both the choke pathogen and the choke fly which could lead to inferences about factors influencing choke disease expression and spread in Oregon orchardgrass.

Materials and Methods

Sampling Design: The study was conducted over two years in the Willamette Valley in western Oregon in cultivated orchardgrass fields within a 10 kilometer radius of the city of Corvallis. In 2008, ten commercial "Potomac" variety seed-production fields of orchardgrass were selected to represent a range of field ages, from 1 to 28 years since original planting. At each field site, five transects were arranged roughly perpendicular to the longest field edge. Along each transect, a grid (1 m^2 quadrat) was placed at 10 locations and were spaced a minimum of 10 meters and a maximum of 25 meters apart, depending on field width. Fifty quadrats were sampled at all field sites. GPS locations were recorded for each quadrat. Within each quadrat, the total number of orchardgrass plants was recorded. Each plant was categorized as being either infected or uninfected based upon the presence of stromata. If infected, the numbers of fertilized and unfertilized stromata were recorded. Each stroma was inspected for choke fly eggs or brood chambers which were counted to estimate fly presence. Transect sampling was repeated in 2009 at four of the sites (site 1, site 2, site 3 and site 4) to examine variability in the following year. Sampling occurred between June 19 and June 29 in 2008, and between June 18 and June 26 in 2009.

Data Analysis: Spatial patterns of fly density per quadrat, stromata per quadrat and symptomatic orchardgrass hosts per quadrat were characterized using Spatial Analysis by Distance Indices (SADIE) (Perry, 1995). The SADIE index, ν, categorizes, for each parameter, individual quadrat locations as above average "clusters" or below average "gaps". Gaps and clusters were mapped using ArcMap (v. 9.3) with the Spatial Analyst extension using the inverse distance weighted method.

To explore the relationship between fly or fungal spatial pattern with change in probability of fungal reproductive success, a logistic regression was performed separately for each site using the following variables: 1) proportion of plants infected per quadrat; 2) the number of stromata per quadrat; 3) the number of flies per stromata; and 4) presence or absence of unfertilized stromata (dependent variable). The presence of stromata was treated as a binary response, with 1 for quadrats with > 0 unfertilized stromata, 0 for quadrats with no stromata.

To assess change between 2008 and 2009 the proportion of plants infected per quadrat, stromata per quadrat, and flies per quadrat were compared separately for each site using the non-parametric Wilcoxon rank sum procedure. Additionally, the age of field was treated as an explanatory variable for the following responses across sites: 1) proportion of infected plants; 2) total stromata; and 3) mean flies per stromata.

Results

Incidence of Choke Disease: In 2008, a total of 3,979 plants were surveyed in 10 orchardgrass seed production fields, of which 1,207 (30.3%) plants were observed to be infected with choke disease. Across all sites, 24,613 stromata were recorded on the hosts, of which only 17 (0.07%) were unfertilized. Unfertilized stromata were found in only 50% of the fields sampled; at sites 3, 5, 6, 7, and 9. In 2009, a total of 1,462 plants were surveyed, of which 328 (22.4%) plants infected with choke. Across the 4 sites included in 2009, 5,338 stromata developed on the hosts, of which only three (0.06 %) stromata were unfertilized. Only one of the three unfertilized stromata was on a plant that did not have fertilized stromata elsewhere. In 2009, all unfertilized stromata were found in site 1.

Abundance of the Choke Fly: In 2008, on the 24,613 stromata recorded, 70,047 choke fly eggs, larvae or brood chambers were recorded, providing evidence of fly visitations (mean =

2.8 flies per stroma). We noted 37 (3.1%) infected plants which had no fly visitation. Across all sites, the number of flies per stroma on each plant varied from 0 to 9.9, with a mean of 2.48 (±S.E. 0.05). There were 215 (17.83% of infected plants) hosts with means of < 1 flies. In 2009, there were 63 (19.2%) infected plants which had no fly presence. Across all sites, the number of flies per stroma on each plant varied from 0 to 5.64, with a mean of 1.39 (±S.E. 0.06). There were 173 (52.7% of infected plants) hosts which had mean fly per stroma density <1.0. The density of flies or stromata did not correspond with the probability of stromata being cross fertilized in 2008, nor in 2009.

Between Year Comparison: Between 2008 to 2009, there was no difference in mean proportion of infected to uninfected plants for site 2, site 3 or site 4. There was an increase in mean proportion of infected to uninfected plants for site 1 from 2008 to 2009. Mean stromata per quadrat also increased at site 1, but not at site 2, site 3 or site 4. There was no mean change in the number of unfertilized stromata per quadrat between 2008 and 2009 for site 1 and site 3. There were no unfertilized stromata found at site 2 or site 4 in either year, so significance tests were unnecessary.

Spatial Characterization: In 2008, the number of plants infected per quadrat was not spatially random, showing distinct clustering in site 2, site 4, site 7 and site 10 (Figures 1 and 2). The number of stromata observed per quadrat showed significant spatial clustering in site 2, site 4, site 5, site 9 and site 10, in 2008. In 2009, two of the four sites sampled showed clustering of infections (site 1 and site 2) (Figure 1). Stromata per quadrat were clustered at site 1, site 2 and site 3, in 2009. In both 2008 and 2009, plots depicted clusters and gaps with variable within-site distributions (Figures 1 and 2). However, between 2008 and 2009, within site locations expressing gaps and clusters tended to remain similar for individual sites 2 and 3, and to a lesser extent sites 1 and 4 (Figure 1).

The age of the orchardgrass stand in fields included in the study ranged from 1 to 29 years. In 2008, the proportion of the field symptomatic with choke disease had no positive trend with field (Figure 3a). Similar results were obtained in 2009. In 2008, the mean number of stromata per infection also did not show a linear relation with age (Figure 3b). Neither was a trend found in 2009.

Discussion

The high level of disease aggregation in orchardgrass fields presents an opportunity to discover factors which might decrease the susceptibility of plants to infection and disease expression. Choke disease symptoms were spatially aggregated at many sites. Moreover, the location of choke disease "gaps" and "clusters" documented that within-field spatial patterns of choke disease expression was fairly consistent across the two years of the study. Abiotic factors (e.g. soil properties), are likely to be aggregated in space within a field (Stafford 2000). Future studies should seek correlations between within-field variation in abiotic variables and expression of choke infection (e.g., by overlaying a soil map on a map of disease expression). If trends are uncovered, these abiotic factors might be manipulated to reduce disease expression in orchardgrass fields.

This study provides evidence that disease spread in cultivated orchardgrass fields occurs principally during the first few years after planting. Site 1, which was planted in 2007, was the only field that showed an increase in choke disease between 2008 and 2009. The other sites surveyed during both 2008 and 2009 were older than site 1, having been planted between 1982 and 1999. We found no correlation between increasing field age and disease incidence or severity of disease expression when including fields of varying age classes (Figure 3a, b). We speculate that certain hosts tend to remain resistant to infection expression across seasons due to abiotic micro-site conditions or plant genetics.

Interestingly, our analysis suggests that fly "pollination" of the choke disease pathogen does not enhance the overall reproductive success of the fungus in western Oregon. We found that while fly density, proportion of plants expressing disease, and stromata density were quite variable between and within each site, fertilization rates of the choke pathogen varied only slightly between and within sites during both years of the study. At all sites, we found almost complete perithecial development on certain stromata without evidence of fly visitation. Neither fly nor stromata density, nor the proportion of plants expressing infection per quadrat correlated with the probability of unfertilized stromata presence. These results correspond with previous observations of Rao and Baumann (2004) and Alderman and Rao (2008).

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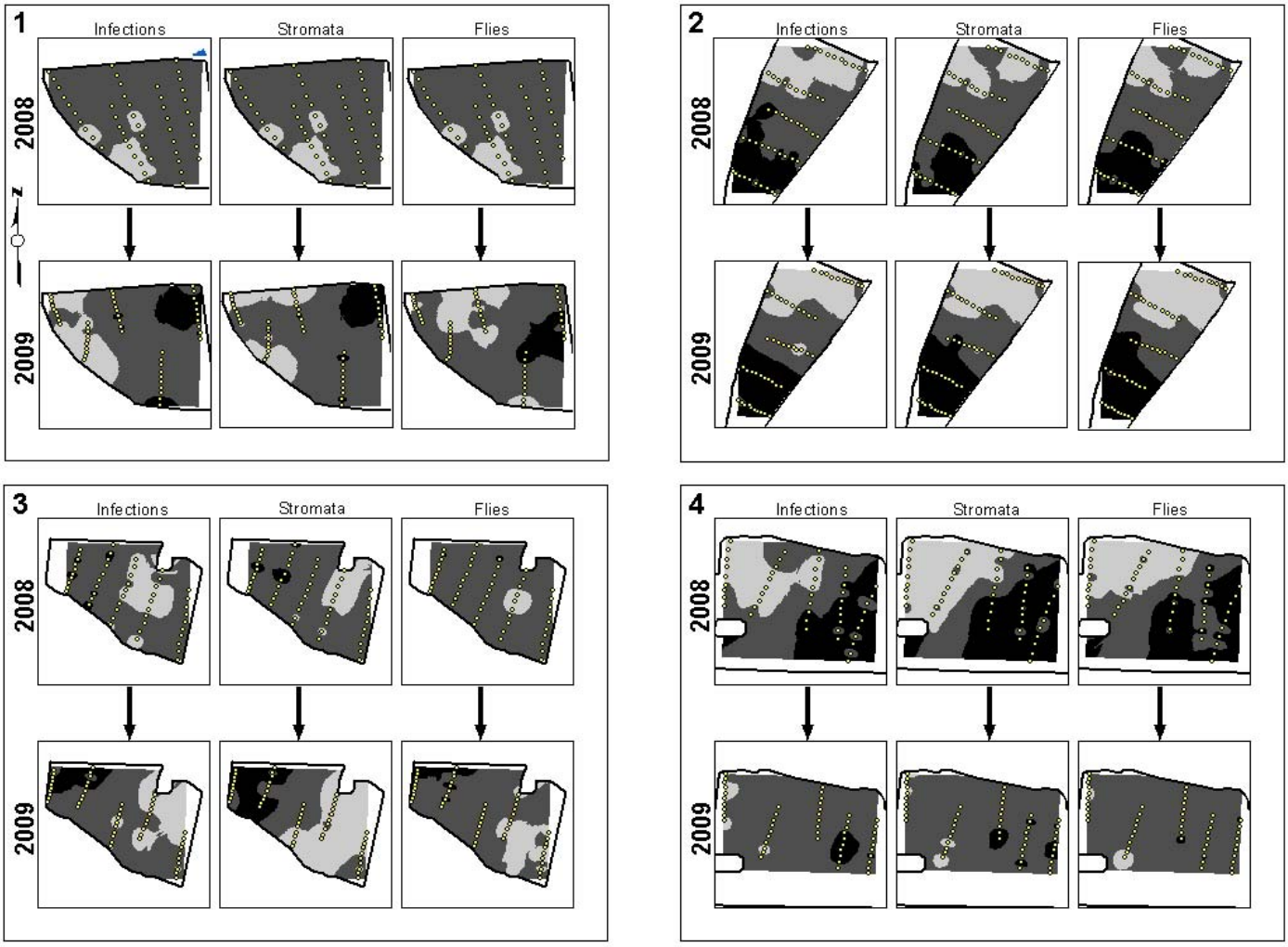

Figure 1. Plots depicting spatial aggregation in sites 1-4 in 2008 and 2009. Within each box, from left to right: spatial pattern of count data for infections per quadrat, stromata per quadrat and flies per quadrat are depicted. Dots represent quadrat sampling locations. Shading represents gaps (light grey), random spatial arrangements (dark grey), and clusters (black). The black lines visible within each box represent field borders.

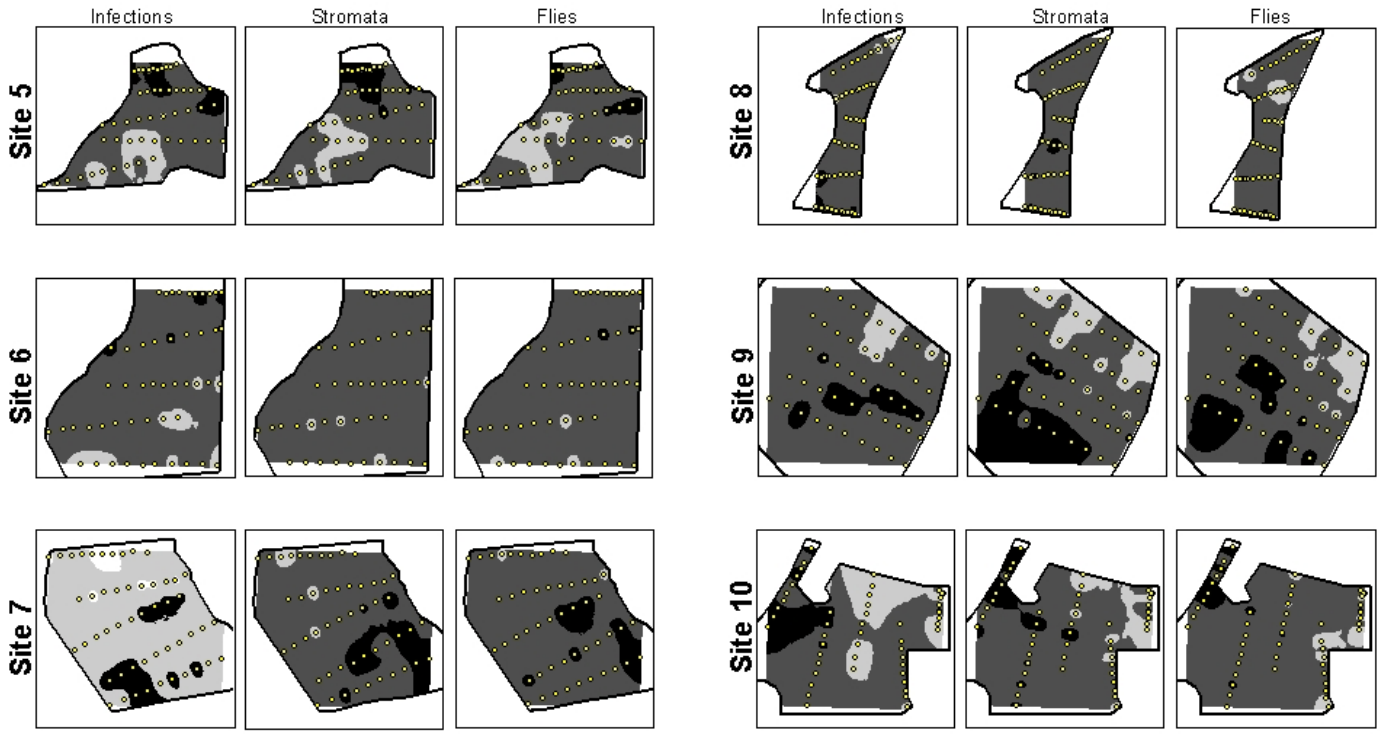

Figure 2. Sites 5 through 10, surveyed in 2008, depicting plots of infections per quadrat, stromata per quadrat, and flies per quadrat. See Figure 1 for further explanation. Six transects were included at site 8, due to an unusually narrow portion of the field which did not allow ten well spaced quadrats.

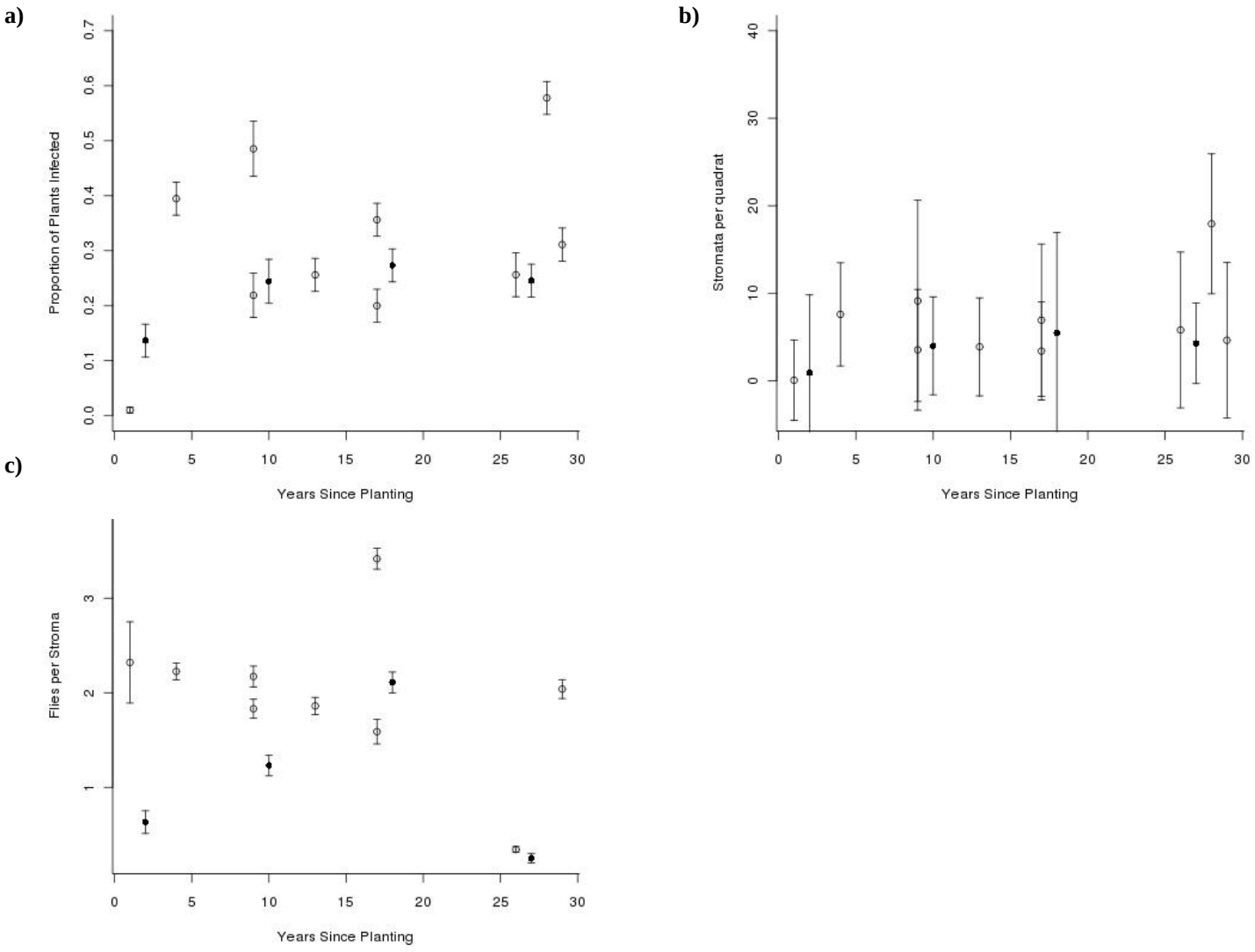

Figure 3. The combined data from 2008 and 2009 for each site plotted against age of field, for: a) Proportion of plants infected in each quadrat; b) Mean stromata per host for each quadrat; and c) Mean flies per stroma for each quadrat. Simple linear regression analysis did not show a linear trend for any of the compared variables ($P>0.05$). Open circles indicate sites from 2008, and filled circles indicate sites from 2009. Standard error bars are depicted.