ENVIRONMENTAL VARIABLES AFFECT FUNGAL DIVERSITY ON BLUEBERRY (*Vaccinium* spp.) LEAF SURFACES

by

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Abstract of the thesis

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ABSTRACT

The economically important blueberry, *Vaccinium cyanococcus,* is susceptible to a number of diseases, some of which are propagated by fungi living on the leaf surface. The leaf surface (phylloplane) is a cryptic environment that harbors a variety of pathogens and pathogen antagonists, and these populations are affected by many factors including weather, season, host plant location and leaf phenology. Blueberry leaves were collected in April, June, August, and October over two years from bushes in wild areas and cultivated farms along transects perpendicular to the Atlantic City Expressway, to address the hypothesis that pollution from a major highway would influence phylloplane communities. Leaves were washed and plated on potato dextrose agar, and fungal epiphytes were identified using taxonomic keys and microscopy. *Epicoccum* spp., *Alternaria* spp., *Pennicilium* spp., and *Curvularia* spp. were the most ubiquitous fungi isolated from blueberry leaves. Community structure and species richness changed from site-to-site and month-to-month and from year-to-year. The influence of highway proximity to fungal communities was not significant. Management practices in cultivated sites accounted for much of the variation in species richness and community composition among sites. Leaf age also influenced the community structure of phylloplane fungi

communities. Leaves collected in April had significantly lower species richness than those collect in later months ($F=19.37$, $P<0.0001$). Yearly differences in species richness and community structure were likely due to differences in meteorological variables. Greater information provided by frequency of occurrence of fungal species would lead to a more informative multivariate analysis as presence or absence would be weighted by abundance, allowing for interpretations of dominance and more detailed analysis of phylloplane fungal communities.

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INTRODUCTION

Vaccinium cyanococcus, the common blueberry, is an economically important species both globally and locally. Hammonton, New Jersey, bills itself as the "blueberry capital of the world". Although this claim may be a bit bold, it demonstrates the importance of blueberry cultivation in the local economy of South Jersey. The highbush blueberry has been commercially important in Philadelphia and New Jersey since at least the mid 1800s (Gough 1994). Intense breeding and selection of wild *Vaccinium* spp. specimens by Elizabeth C. White in the early 1900s allowed the production of favorable varieties that could be transplanted and cultivated. Many crosses have been made between different cultivars to produce plants with specific qualities and tolerances for specific soil conditions and climates. The modern cultivated highbush blueberry, *Vaccinium corymbosum*, is a result of such a cross (Gough 1994). The United States Department of Agriculture (USDA) breeding center in Chatsworth, NJ is studying this increased diversity to ensure an increase in berry quality and disease resistance (Gough 1994).

A wide variety of pathogens including viruses, nematodes, and fungi can infect and damage blueberry bushes (Eck 1988). Fungi compose the majority of blueberry plant pathogens, and can infect leaves, stems, roots, blossom and fruit (Eck 1988). Many diseases are cultivar specific (Gough 1994), although the fungal pathogen *Monilina vaccinii-corymbosi* (mummy berry disease) can affect multiple cultivars to different degrees (Eck 1988). *Monilina vaccinii-corymbosi* ascospores germinate on the surface of leaves and blossoms leading to infection (Eck 1988). This disease is a problem for many blueberry farmers and biological controls have been developed that utilize bees as a

vector to deliver a strain of *Bacillus subtilis* to reduce *M. vaccinii-corymbosi* infection (Dedaj et al. 2004). *Bacillus subtilus* QST 713 (marketed as QST 713 or Serenade™) produces antifungal compounds thus aiding in controlling *M. vaccinii-corymbosi* on the leaf and blossom surface preventing infection of the plant (Dedej et.al. 2004).

Leaf surfaces: the phylloplane

The leaf surface, also called the phyllosphere or phylloplane, is an important substrate for microbial life. Numerous studies have examined bacterial (Hirano & Upper 2000, Jurkevitch & Shapira 2000) and fungal (Osono et. al. 2004) presence (Osono & Mori 2004) and processes (Ruinen 1970) on the surface of leaves. Many factors, including, but not limited to differences and changes in leaf phenology, climate and microclimate, leaf position, species, surrounding flora and fauna, and ecological interactions (i.e., competition, predation) influence the diversity and biomass of phylloplane microbial community (Lindlow & Brandl 2003, Lindow & Leveau 2002, Kinkel 1997).

The phyllosphere is considered a hostile environment in that water availability, insolation, and nutrient availability change regularly (Lindow 2002). Climate and meteorological cycles influence the phyllosphere and can cause vast differences in temperature, available moisture and evaporation rate in short time (Hirano & Upper 2000). Weather systems and seasons impose even longer term changes on the phylloplane (Hirano & Upper 2000). Leaf morphology and chemistry affect the size of microbial phyllosphere populations (Yadav et al. 2005). As the leaf ages, time passes allowing more microbes to accumulate, and it grows larger, creating more substrate for microbial biomass. The surface texture also becomes increasingly rough as the plant ages

(Mechaber et al. 1996), possibly as a result of microbial action (Knoll & Shrieber 2000) thus altering the substrate even more. Time and physical changes further influence phylloplane microbial populations.

Although the microbiology of roots has been studied more than the phylloplane, it can be argued that the phylloplane is of even greater importance than the rhizosphere because of the potential for controlling agricultural disease (Lindow & Leveau 2002). Morris and Kinkel (2002) propose that phyllosphere microorganisms could be used as biocontrols and alter the leaf surface habitat and disrupt pathogen activity on leaf surfaces. An estimated 10^{26} bacterial cells are thought to live on leaf surfaces globally and Morris and Kinkel (2002) suggest these bacteria could influence host plant behavior and possibly global biogeochemical cycles.

The phylloplane is where light, water and nutrients exchange between the environment and the plant. Phylloplane microbial communities are important in the uptake of inorganic nitrogen and other nutrients from atmospheric deposition. Abril (2004) mentions that nitrogen fixation in the phyllosphere may be the primary means for nitrogen uptake in humid tropical ecosystems. Vegetation surrounding the host plant and microbial populations on the phylloplane itself (Stohr & Dighton 2004) can influence the communities on the leaf surface. Stadler and Michalzik (1999) showed that the presence of aphids and their resulting honeydew, a source of energy for phylloplane microbes, increased microbial populations by nearly 100-fold.

The surrounding environment including local flora influences atmospheric microbial concentration and composition at a given location geographically (Bovalius et al. 1978), and on trees (Osono & Mori 2004). This will dictate what type and numbers of

microbes settle and grow on the phylloplane (Lindemann & Upper 1985). Also, position on the tree itself influences microbial populations with lower levels receiving less variation of abiotic factors creating more stable environments for microbes (Kinkel 1997). Still other studies indicate that shoot age may play a part in influencing phylloplane communities (Osono & Mori 2004).

Effects of pollutants on phylloplane communities

Anthropogenic toxins such as heavy metals and automotive exhaust were observed to affect phylloplane communities (Kul'ko & Marfenina 2001). Zinc, lead, and cadmium reduced phyllosphere microbial abundance and diversity of contaminated cabbages and pine saplings when compared with their non-contaminated counter parts (Gingell et al 2003). Studies from the mid 20th century have noted how sulfur dioxide and ozone can injure leaves (Robinson 1971). Heavy metal poisoning and carbon monoxide associated with heavily trafficked roads in Moscow influenced the population of microorganism in soil and surface air (Kul'ko and Marfenina 2001). More studies in India found phylloplane fungal distributions were influenced by air pollutants from automobiles and some fungi could be used as bioindicators of air pollution (Niwas et al. 1988).

Cultivated crops use management practices such as the application of pesticides and fungicides that further alter phylloplane communities. Studies in New Zealand showed organic apple farms had higher numbers and species richness of phylloplane microbes than managed sites (Waipara et al. 2002). Although this study shows a difference in phylloplane communities with different management practices, it did not compare wild plants in a heterogeneous environment with cultivated monoculture stands

Numerous studies have documented the phylloplane community and the processes that determine their presence and patterns (Hirano & Upper 2000, Jurkevitch & Shapira 2000, Osono et al. 2004, Osono & Mori 2004, Ruinen 1970), however data regarding phylloplane communities of wild or cultivated blueberries is lacking. Automotive exhaust does influence phylloplane communities; however data regarding the distance to which automotive exhaust will influence phylloplane fungi are sparse. New Jersey is the most densely populated state in the United States with an estimated 6.3 million vehicles registered in state (Bureau of Transportation Statistics) and data regarding the effect of automobile exhaust on phylloplane communities of blueberries are lacking.

Objectives

The object of this study is to sample fungal phylloplane diversity on wild and cultivated highbush blueberries (*V. corymbosum*) along a transect perpendicular to the Atlantic City Expressway over two growing seasons to examine: 1) how phyllosphere fungal community compositions and species richness compare among wild and cultivated individuals of *Vaccinium* spp. and 2) how seasonal and climate variability over two years affects these patterns and 3) how distance from a source of pollutants derived from motor vehicles impacts phylloplane fungal species richness and community compostion.

Wild and cultivated blueberries will harbor different phylloplane communities (Hypothesis 1). Studies by Waipara et al. (2002) have shown differences in phylloplane community structures in cultivated apple orchards under different management techniques. Since differences in management techniques yielded differences in phylloplane community structure, wild, or unmanaged blueberry bushes should harbor

different phylloplane communities than their irrigated and pesticide- and fungicide-treated relatives.

Seasonal changes in the biophysical properties of leaf surfaces occur and fungal phylloplane community structure will also change as the growing season progresses (Hypothesis 2). Bakker et al. (2003) demonstrated seasonal changes in phylloplane community density and structure of apple trees as well. Distance from the highway could also have an affect on phylloplane community structure with phylloplane communities closer to heavily traveled roads having lower diversity than those at a greater distance (Hypothesis 3), as noted on ornamental plants by Niwas (1988).

Finally, it is likely that the surrounding forest of wild sites altered the transport and deposition of pollutants derived from automotive exhaust as distance from the highway increases when compared with open monoculture-managed sites. Studies by Bovallius et al. (1978) have noted how surrounding vegetation affects airborne microbe concentrations. Other studies (Lindemann et al. 1982) have noted that surrounding plants can be sources of airborne microbes. Studies by Lindemann et al. (1985) have shown that surrounding vegetation and the resulting air currents can strongly influence phyllosphere microbe populations. Findings from these studies suggest that the surrounding canopy structure (or lack of canopy) near wild blueberries will affect the phylloplane community, causing dissimilar compositions when isolations from wild blueberry leaves are compared with those populations isolated from the cultivated sites.

MATERIALS AND METHODS

Study sites

Study sites were in the Pine Barrens of southern New Jersey, the largest uninterrupted forest on the eastern coastal plain which encompasses over one million acres, approximately 22% of New Jersey (McCormick and Jones 1973). This unique ecosystem experiences a cool temperate climate with a January mean of 0.3ºC and June mean of 23.8ºC, and precipitation averages 1,123 ± 182 mm (State Climatologist of NJ), and is frequented by high intensity wildfires (Foreman 1981).

Lowland forests occupy 38% of the forested areas in the Pine Barrens and are dominated by three major forest communities: Pitchpine lowlands, Atlantic white cedar stands, and mixed hardwood swamp (McCormick and Jones 1973). The understory of these communities is composed of ericaceous shrubs such as huckleberry (*Gaylussacia bacata, G. frondosa*) and blueberry (*Vaccinium* spp.). *Smilax spp*., *Clethra alnifolia*, sedges, sphagnum mosses and lichens are also present.

The Atlantic City Expressway (ACE) was selected as a possible point source of pollution within the forest. The ACE is a multilane highway that connects the Philadelphia area with Atlantic City and other shore points. More than 66 million vehicles travel through its tollbooths annually with an estimated 16 million vehicles between the late May holiday of Memorial Day and Labor Day in the beginning of September, when blueberries are in full leaf (South Jersey Transportation Authority). See Appendix 1 for exact figures relating to highway traffic through the study site broken down by month and nearest toll booth.

Wild site

Wild sites were selected in lowland forest, dominated by mixed pines and hardwoods. Understory vegetation included *smilax spp*., *Clethra alnifolia* and patches of sphagnum directly under the last two bushes sampled. Two adjacent transects perpendicular to the ACE 100 meters south were used to select bushes. (see Appendix 2) Bushes from five points along the transect at 1) 39º41'08.3" Latitude (Lat) 074º54'44.9" Longitude (Long), 2) 39º41'07.7" Lat 074º54'45.6" Long, 3) 39º41'07.4" Lat 074º54'46.3" Long, 4) 39º41'07.0" Lat 074º54'46.9" Long, and 5) 39º41'06.4" Lat 074º54'47.4" Long were sampled. Samples consisting of an individual leaf were taken from outer mid level, west facing leaves from two adjacent bushes every 20 meters or bushes as close to these points as possible. Transects were in the middle of a forest patch (100 meters either side) to ensure homogeneity and minimize forest, edge, canopy, or other effects on sampling.

Cultivated Site

Two adjacent transects perpendicular to the ACE extended for 100 meters north from the ACE (see Appendix 2). Bushes from five points along the transect at 1) 39º36'33.1" Lat 074º49'13.4" Long 2) 39º36'33.6" Lat 074º49'12.9" Long 3) 39º36'034.2" Lat 074º49'12.4" Long 4) 39º36'34.9" Lat 074º49'12.1" Long 5)39º36'34.9" Lat 074º49'11.1" Long were sampled. Samples consisting of an individual leaf were taken from outer mid level, west facing leaves from 2 adjacent bushes every 20 meters or bushes as close to these points as possible. Transects were perpendicular to bush rows and located in the middle of the field to ensure homogeneity and minimize forest, edge, canopy, or other effects on sampling. These bushes were

subjected to an agricultural chemical regime (see list of dates and application rates, Appendix 3).

Collections procedure and dates

Leaves were collected on 10 dates: late April, late June, early August and early October. Cultivated leaves were first collected on April 18 (shortly after bud break) and wild leaves were not collected until April 28 as the wild sites had a later bud break. The remaining 2006 samples were collected on June 22, August 21 and October 10. High rainfall and cool cloudy days delayed bud break in 2007 and cultivated leaves were first collected April 23 whereas wild leaves were not collected until May 6, 2007. The remaining 2007 leaves were collected on June 21, August 22, and October 11. Leaves were collected from designated locations and placed in pre-labeled plastic bags with a zipper-like closing mechanism.

Epiphyte isolation

Leaves were transferred from the plastic collecting bag within two hours and placed in a 250 mL Erlenmeyer Flask with 150 mL of sterile de-ionized water on an orbital shaker for (170 rpm) for 1 hour prior to transfer to Potato dextrose agar. Leaves were left on PDA agar plates for four to six days to let mycelia grow from leaves onto media. When colonies were visible, fungi were aseptically transferred into pure culture for further identification and classification. Fungal identification keys (Wang & Zabel 1990, Caruso & Ramsdell 1995) and light microscopy were used to identify fungal taxa through spore recognition. Fungi which could not be identified were placed in morpho-groups based on macro- and micro-scopic characteristics allowing for others unknown

fungi to be placed accordingly. Marshall Bergen at Rutgers Cook Campus was instrumental in identifying some of the more difficult groups.

Meteorological data

Meteorological data, specifically mean temperature, precipitation, relative humidity, and solar radiation were recorded by the United States Forest Service at the Cedar Bridge weather station approximately 45 to 48 kilometers away from the study sites. Monthly means for these factors were used to compare seasonal and annual changes in weather relating to phylloplane habitat stability (see Appendix 4).

Data analysis

Data were organized in Excel (Microsoft Software 2002) in binary format (e.g. presence/absence). Leaf site, position, and collection date were listed vertically while the species and morphotypes ran horizontally across the top, and the grid was filled in accordingly with a "1" representing presence and a "0" representing absence. Difference between fungal community composition of the treatments, and dates was assessed by Principle Component analysis (PCA) using PC-ORD (MjM Software Design 1999). Euclidian distances between coordinate scores between treatments were analyzed by Analysis of Variance, ANOVAs with SAS (SAS Inc. 1990) software. Significantly different contrasts were detected using Tukey's mean significant difference, (MSD) test.

Statistical tests

A priori tests consisting of ANOVA's comparing species richness and community composition in the form of coordinate scores of PCA's were completed to examine differences between site, season, and position relative to highway. A posteriori

tests comparing year to year differences in richness and community composition were also completed.

RESULTS

Fungal populations

Between April 2006 and October 2007, 52 genera or morphotypes of phylloplane fungi were isolated from *Vaccinium* spp. The most ubiquitous fungi was *Epicoccum* spp. followed by *Alternaria* spp., *Pennicilium* spp. and *Curvularia* spp., all of which appeared in at least 40% of the leaves sampled (Table 1).

Species richness and species composition changed seasonally, annually and from site-to-site and there were also yearly differences between months as well. Multiple Principle Component Analyses (PCA) were performed on the communities based on site, date, and location. Overall, site and date (seasonal and year to year) had the strongest effect on community composition. ANOVAs of the component scores from the PCA of the fungal communities indicated that these two variables generally accounted for observed patterns.

Species richness

Cultivated 2006 samples were significantly different in species richness among months ($F=19.37$, $P<0.0001$; Appendix 5) when tested using an ANOVA. A Tukey MSD test showed leaves collected in April harbored significantly less diverse communities than leaves collected during other sampling periods (Table 2). August communities had the highest biodiversity, although they were not grouped separately from June and October communities by a Tukey test.

Species richness data showed a significant position by month interaction ($F=2.29$, $P=0.049$; Appendix 5). Wild 2006 leaves showed a trend toward a difference in species richness among monthly treatments ($F=2.65$, $P=0.076$; Appendix 5). Although not

statistically significant, it followed the trend seen in the fungal phylloplane communities of cultivated shrubs.

Cultivated 2007 monthly treatments had a significantly different species richness among months ($F=10.23$, $P=0.0003$; Appendix 5). April had the lowest diversity, June was intermediate, and August had the highest diversity whereas October showed the second highest diversity of phylloplane fungi (Table 3). Leaves collected in 2007 from the cultivated site had similar seasonal patterns in species richness as those found in the 2006 cultivated leaves. This general increase in richness follows the termination of an agricultural chemical application regime that ends in July, with the exception of a final spraying of an insecticide for *Scaphytopius magdalensis* (sharp nose leaf hopper) eradication in September. A seasonal difference in species richness was detected on wild samples ($F=6.70$, $P=0.0026$; Appendix 5) but there was no clear pattern (Table 4). Species richness of wild communities was highest in June of both years, although only the 2007 data showed a significant difference.

Cultivated leaves for all sampling events showed a seasonal effect in species richness ($F=24.08$, $P<0.0001$; Appendix 5) with April being the least diverse, followed by June, then August and October being similar to each other (Table 5). Species richness averages for leaves collected in the wild for all sampling events also showed seasonal differences in species richness ($F=6.53$, $P=0.0011$; Appendix 5), but no patterns were discerned (Table 6).

Annual community analysis for 2006

PCA for combined 2006 (Figures 1 and 1a) samples showed seasonal and site differences in fungal communities. Axes one and two combined accounted for 28% of

the variance in the communities. April and June samples both clustered and had similar community compositions anchored by *Alternaria* spp., *Aureobasidium* spp., and *Epicoccum* spp. along axis one. Along axis two, these two months separated, with April samples having *Penncilium* spp. as a prevalent community member and *Curuvularia* spp. being more established in the June communities. The frequency of *Curvularia* spp.and *Nigrospora* spp. in October fungal communities separated these samples from June and April communities along axis one. August communities were spread throughout the data set and did not cluster; however, wild August fungal communities had more in common with October communities, whereas cultivated August data points were clustered near the June points, indicating a similarity.

Axis one apparently followed a time progression, with early communities on the left (Fig. 1 and 1a) containing *Alternaria* spp., *Aureobasidium* spp., *Epicoccum* spp. and later communities on the right harboring *Curvularia* spp*., Nigrospora* spp., and RP. ANOVAs of the coordinate scores revealed significant differences in fungal communities among months ($F=42.62$, $P<0.0001$; Appendix 5), with each month having a separate Tukey (Tukey MSD) grouping test along axis one (Table 7). A significant site by month interaction also existed ($F=11.85$, $P<0.0001$; Appendix 5).

The 2006 data set indicated a trend of highway effect, with a P value of 0.0789 (Appendix 5) along axis two. Axis two showed significant site ($F=16.70$, $P=0.0002$; Appendix 5) and month ($F=13.25$, $P<0.0001$; Appendix 5) differences, with a site by month interaction ($F=5.30$, $P=0.0036$; Appendix 5) as well. June, August and October communities were grouped similarly with a Tukey MSD test, leaving April's communities different than the other months (Table 8).

Annual community analysis for 2007

Leaves collected in 2007 (See Figures 2 and 2a) showed differences in fungal communities depending on site and month. The first two axes on the PCA accounted for 36% of the variance. Wild and cultivated samples collected in April 2007 had communities that were somewhat similar to each other and were clustered in one quadrant of the PCA analysis. June, August, and October communities varied and did not form a discernable pattern. An ANOVA of the coordinate scores showed a significant difference between sites ($F=9.37$, $P=0.0039$; Appendix 5) and months ($F=14.98$, $P<0.00001$; Appendix 5) along axis one. Axis one also had a significant site by month interaction ($F=6.80$, $P=0.0008$; Appendix 5). April and June communities were clustered, but these communities were significantly different than the August and October samples, which were similar to each other (Table 9). Axis two also had a significant difference between sites ($F=52.45$, $P<0.00001$; Appendix 5) and months ($F=11.97$, $P<0.00001$; Appendix 5). In addition, axis two showed a significant site-by-month interaction ($F=6.54$, $P=0.0010$; Appendix 5). June, August and October communities were grouped similarly with a Tukey MSD test (Table 10) with April's communities different than those of the other months.

Monthly comparisons demonstrating site and annual differences

April

Figure 3 shows a principle component analysis of April samples from 2006 and 2007. The first two axes combined to account for 46% of the variance in the populations and showed differences in fungal communities among sites and years. ANOVA performed on the coordinate scores of the PCA showed a significant difference in fungal

communities among sites along axis one (F=15.70, P=0.0003; Appendix 5). This difference was driven primarily by *Alternaria* spp., *Aureobasidium* spp. and *Pennicilium* spp. in the cultivated sites and morphogroups Gs2, #1, and *Trichoderma* spp. in the wild sites. Note the PCA created no negative eigen-values for axis one and the lowest values were used to form components for the negative end of axis one. Figure 3a shows a tightly clustered group of cultivated sites along axis one whereas the wild sites are spread out along both axes. Axis one had a difference among years (F=9.75, P=0.0035; Appendix 5) in addition to the difference in site. This difference may be due to large differences in the meteorological variables among years for April in the Pine Barrens; April 2007 mean air temperature was 2.5ºC lower, days were cloudier, and precipitation was 85% greater when compared with April 2006 (Appendix 4). Axis two exhibited no significant differences in site or year. April fungal species richness of wild (F=13.09, P=0.0047, Appendix 5) and cultivated (F=15.88, P=0.0002, Appendix 5) blueberry shrubs varied significantly between 2006 and 2007.

June

A PCA of June 2006 and 2007 samples indicated significant differences in fungal community composition among treatments and years, with the first two axes accounting for 41% of the variance. Figure 4 demonstrates fungal communities isolated from June samples. Axis one had a difference community structure among wild and cultivated sites as indicated by an ANOVA of the coordinate scores (F=32.40, P<0.0001; Appendix 5) and an annual difference as well. (F=33.21, P<0.0001; Appendix 5). Cultivated communities were dominated by *Alternaria* spp., *Epicoccum* spp. and Penn White. *Pennicilium* spp, BGP, and *Phyllostica* spp., a known blueberry pathogen (Carusoe and

Ramsdell 1995) dominated community structure in the wild sites. Although no significant difference in communities among sites was found along axis two, a significant difference (F=6.05, P=0.0189; Appendix 5) in fungal communities existed between years. Meteorological factors could again be partially responsible with June 2007 receiving greater insolation, less rain, and slightly higher temperatures when compared with June 2006 (Appendix 4). Axis two revealed a site-by-year interaction (F= 4.25, P=0.0465; Appendix 5). Species richness of cultivated June samples varied annually (F=5.00, P=.0493, Appendix 5).

August

An ANOVA of the coordinate scores from the PCA indicated fungal communities from August 2006 and 2007 showed differences among treatments and years on both axes (site axis one; F=71.18, year axis one: F=33.04, site axis two: F=21.44, year axis two: F=43.32, all P values <0.0001; Appendix 5). Axes one and two accounted for 23.5% and 14.5% of the variance, respectively. As seen in Fig. 5, *Alternaria* spp. was prevalent in the communities of cultivated leaves collected in 2006, and absent in the wild sites from both years. Wild August samples were separated from the cultivated samples along axis two with *Curvularia* spp. and *Trichoderma* spp. comprising key components of the wild sample's fungal community while the cultivated sites were influenced by *Alternaria* spp. and FC2. Although *Alternaria* spp. was a major component of the 2006 communities, *Pennicilium* spp. was the dominant species in the 2007 communities.

October

The first two axes of the PCA accounted for 33% of the variance and coordinate scores subjected to ANOVA showed October 2006 and 2007 (See Figure 6 and 6a) samples had a difference in community structure among sites ($F= 8.43$, $P=0.0063$; Appendix 5) along axis one. Figure 6a better illustrates the separation of sites along axis one. While cultivated communities had been dominated by *Curvularia* spp. and *Nigrospora* spp., wild communities had more of the morphogroups Penn White and White Matt. Both wild and cultivated communities' structure means in 2007 shifted down and towards the left on the graph relative to their 2006 positions. Axis two showed a difference among sites ($F=8.18$, $P=0.0070$; Appendix 5) and years ($F=60.10$, $P<0.0001$; Appendix 5). *Epicoccum* spp. and *Trichoderma* spp. were seen to drive the community structure of wild type communities whereas *Nigrospora* spp. and *Alternaria* spp. were more frequent in the cultivated groups. Again, meteorological variables could have had an effect on this difference, as fall in 2007 was much warmer and drier when compared with 2006 (Appendix 4). Cultivated samples were separated along axis 2 with *Epicoccom* spp. and *Trichoderma* spp. more prevalent in the 2007 communities and *Nigrospora* spp. and *Alternaria* spp. forming key components of the cultivated 2006 communities.

DISCUSSION

Phylloplane fungal communities exhibited differences in composition and richness throughout the study period. Differences in meteorological variables likely influenced the annual species richness differences in April and June as well as annual differences in community structure in all months sampled. Management practices influenced the richness and composition of fungal phylloplane communities of leaves from cultivated shrubs when compared with their wild counterparts. The seasonal differences among communities did not demonstrate clear community progression patterns however, some individual species exhibited notable seasonal patterns.

The data did not support the initial hypothesis of highway proximity affecting phylloplane community structure or species richness. However, the 2006 data indicated a significant position by month interaction in species richness (P=0.0049; Appendix 5). This highway effect was combined with a seasonal effect, so a true highway effect was not apparent. Although axis two of the PCA for 2006 data indicated a trend in highway effect in community composition, the confidence interval was not strong enough to state that highway proximity had an effect on phylloplane fungal communities. Studies in India (Niwas et al. 1988) showed highway proximity had an effect on fungal populations of leaf surfaces whereas studies in Moscow (Kul'ko & Marfenina 2001) showed highways had an effect on soil and surface air bacteria. The differences in emission control regulations among countries, with the US and NJ restrictions (NJ DEP Low Emission Vehicle regulation) more stringent than those of other countries, could account for the muted highway effects on the fungal communities observed in this study.

However, sites, months and years exhibited differences in richness and community structure.

Annual differences

Differences in community structure and richness between years within months could be due to annual differences in rainfall or other climatic factors. Bud break in 2007 was approximately 7 to 10 days later than in 2006. This followed a cool wet period in April with low sunlight, 86% more precipitation, and a mean temperature 2.7˚C cooler than April 2006. This weather pattern was noted by blueberry farmers and documented in the *Atlantic City Press* (Press of Atlantic City). Annual variations in meteorological factors also affected surrounding phylloplane communities, further altering community structure of leaves samples by changing the surrounding immigration sources for phylloplane fungi.

The later bud break in 2007 may explain the difference in wild and cultivated communities' species richness between years for leaves collected in April. There was also a difference in June cultivated samples species richness between years. This and differences in community structure between cultivated 2006 and cultivated 2007 samples could be due to the small differences in the agricultural chemicals applied from year to year and in that the cultivated plants were all of a single variety. Spray records from 2006 were unavailable, however 2007 and 2008 spray records showed differences between each year's spray regimen. Small differences in chemicals and application date, coupled with slightly different annual collection dates could account for changes in communities between years of seasonally early cultivated communities.

Site differences

Differences in community composition and richness between sites were likely due to the regime of agricultural chemical applied to the cultivated fields. Ten to 15 applications of pesticides are applied annually over a period of four months starting in April and ending in July, with a final spraying for *S. magdalensis* (sharp nose leaf hopper) in September.

Pesticide application could also be responsible for the homogenous nature of cultivated communities clustered around axis one in April 2006 and 2007 samples. *Phyllosticta* spp., a blueberry pathogen (Caruso and Ramsdell 1995) *Trichoderma* and *Curvularia* spp. were absent from June cultivated samples but found throughout June 2006 and 2007 in wild blueberry phylloplane fungal communities. *Phyllosticta* spp. was likely a target species of the fungicide application, while the loss of *Trichoderma* spp. and C*urvularia* spp. could have been collateral damage caused by the use of agricultural chemicals.

Further differences in richness and composition between sites could be due to microclimatic factors as influenced by the presence of a forest canopy or the surrounding heterogeneous understory in the wild blueberry, compared with the homogenous blueberry farm. Studies by Bovallius et al. (1978), Osono & Mori (2004) and Stohr & Dighton (2004) indicate that location and surrounding vegetation can affect the types of microbes that are able to colonize a particular area.

The presence of gypsy moths (*Lymantria dispar*) and the effects of leaf damage could have also influenced wild site populations by creating less leaf area to exist. Leaf damage via herbivory likely altered leaf surface chemistry and changed fungal

phylloplane communities. Some leaves were heavily damaged with over approximately 50% of the leaf area consumed by gypsy moths. This missing surface area and altered leaf chemistry may have affected species richness and composition.

Seasonal differences

The application of fungicides also apparently influenced the seasonal changes seen in species richness and fungal community composition from cultivated leaves. When the pesticide application ended, community structure was observed to change, and a notable increase in species richness occurred both years. Species richness peaked in cultivated samples in August of both years, although only significantly higher than other months in 2007. Species richness of wild leaves peaked in June, earlier than the cultivated communities. The presence of fungicides and other agricultural chemicals on the early season cultivated communities likely caused the fungal population to peak later on cultivated leaves than on wild leaves.

These applied chemicals disturbed equilibrium and altered the normal composition of undisturbed wild type phylloplane fungal communities. The conclusion of the fungicide application ended an environmental stress and created a vacuum in the phyllosphere community. This vacuum filled with competing fungal species that eventually reach equilibrium. This fits with the ecological theory of Grime (1977) in which the community of phylloplane fungi is restricted to stress tolerant species under the pesticide regime and being released on the removal of pesticide application to contain more ruderal or combative species.

A seasonal change in fungal communities of wild leaves was noted in both years. Fungal community species richness on wild shrubs peaked in June while the cultivated

communities peaked in August. As the leaf aged, new chemical resources (Yadav et al. 2005) became available and physical spaces formed (Mechaber et al. 1996), allowing fungi to compete, exploit and dominate their particular new niche. Also, the presence and effects of gypsy moths in June both years could have exacerbated an environmental stress by reducing colonization area and thus changing fungal community structure even further.

In addition to the general patterns observed, some fungi followed patterns that proved significant. *Aureobasidium* spp. was far less prevalent in the second year during which there was higher rainfall. This fits with studies by McCormack et al. (1995) that showed *Aureobasidium* spp. was more efficient at combating competitors via biocides in drier conditions. This species was not present in any late samples but was present in wild and cultivated April and June samples.

Alternaria spp. was absent from the phylloplane fungal communities collected from August wild leaves of both years. Gypsy moth predation had affected leaf surface area and chemistry, and could have been responsible for creating inhospitable conditions for *Alternaria* spp. to survive. Leaves could have exuded chemicals as a result of the gypsy moth predation which may be responsible for the elimination of *Alternaria* spp.

In conclusion, phylloplane fungal community structure and richness were not significantly affected by highway proximity. Further studies that address highway effects on phylloplane fungi could benefit from greater sample size, being closer to a higher volume highway, or a highway in a locale that has less stringent emission controls. Perhaps this increased source of pollutants would have a noticeable effect on leaf surface biota. Although no significant highway effect was determined, this study demonstrated

differences in community structure and richness based on site and season. These patterns could generally be explained by environmental and meteorological factors.

Future studies could benefit from establishing frequency of fungal species occurrence instead of the presence and absence binary format. Having numerical data on frequency of occurrence would allow measures of diversity and evenness of the community to be calculated, providing more information than the species richness *per se*. Additionally, the greater information provided by frequency of occurrence of fungal species would have led to a more informative multivariate analysis as presence or absence would be weighted by abundance, allowing for interpretations of dominance and more detailed analysis of these cryptic environments.

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Table 1. Frequency of occurrence of the most common phylloplane genera on both natural and cultivated blueberry over both years.

Species	Number of occurrences (xx/160)
Epicoccum	69
Alternaria	65
Pennicilium	65
Curvularia	64
Aureobasidium	25
Nigrospora	22
Fusarium	20
Trichoderma	18
Wisp	17
Phyllosticta	17
FC2	15

Table 2: Monthly phylloplane fungal species richness for commercial blueberries in 2006, showing the Tukey grouping, the means and standard error (SE).

Month	Mean ± S.E.	Group
April	1.3 ± 0.4955	B
June	3.1 ± 0.4984	A
August	4.2 ± 0.5429	A
October	3.8 ± 0.5003	A

Table 3: Monthly phylloplane fungal species richness for commercial blueberries in 2007, showing Tukey grouping.

Month	Mean ± S.E.	Group
April	0.6 ± 0.267	C
June	2.1 ± 0.277	C B
August	4.4 ± 0.452	A
October	3.5 ± 0.703	AB

Table 4: Monthly phylloplane fungal species richness for wild blueberries in 2007, showing Tukey grouping.

Month	Mean ± S.E.	Group
April	1.7 ± 0.448	B
June	5.3 ± 0.559	A
August	2.9 ± 0.605	B
October	3.5 ± 0.342	AB

Table 5: Monthly phylloplane fungal species richness for cultivated blueberries in 2006 & 2007, showing Tukey grouping.

Month	Mean ± S.E.	Group
April	0.95 ± 0.285	C
June	2.6 ± 0.234	B
August	4.3 ± 0.282	A
October	3.65 ± 0.365	AB

Table 6: Monthly phylloplane fungal species richness for wild blueberries in 2006 & 2007, showing Tukey grouping.

Month	Mean ± S.E.	Group
April	2.9 ± 0.458	B
June	4.85 ± 0.509	A
August	2.6 ± 0.380	B
October	3.3 ± 0.333	B

Table 7: 2006 Phylloplane fungal community composition analysis: PCA axis one, showing Tukey grouping.

Month	Mean ± S.E.	Group
April	-0.1929 ± 0.099	C
June	-0.6009 ± 0.114	D
August	0.1929 ± 0.099	B
October	0.6000 ± 0.0671	A

Table 8: 2006 Phylloplane fungal community composition analysis: PCA axis two, showing Tukey grouping.

Month	Mean ± S.E.	Group
April	0.461 ± 0.073	A
June	-0.1717 ± 0.082	B
August	-0.0015 ± 0.137	B
October	-0.2876 ± 0.134	B

Table 9: 2007 Phylloplane fungal community composition analysis: PCA axis one, showing Tukey separation.

Month	Mean ± S.E.	Group
April	-0.4396 ± 0.073	B
June	-0.2809 ± 0.122	B
August	0.499 ± 0.162	A
October	0.2214 ± 0.134	A

Table 10: 2007 Phylloplane fungal community composition analysis: PCA axis two, showing Tukey separation.

Month	Mean ± S.E.	Group
April	-0.3992 ± 0.032	B
June	0.0222 ± 0.138	A
August	0.0915 ± 0.146	A
October	0.2855 ± 0.109	A

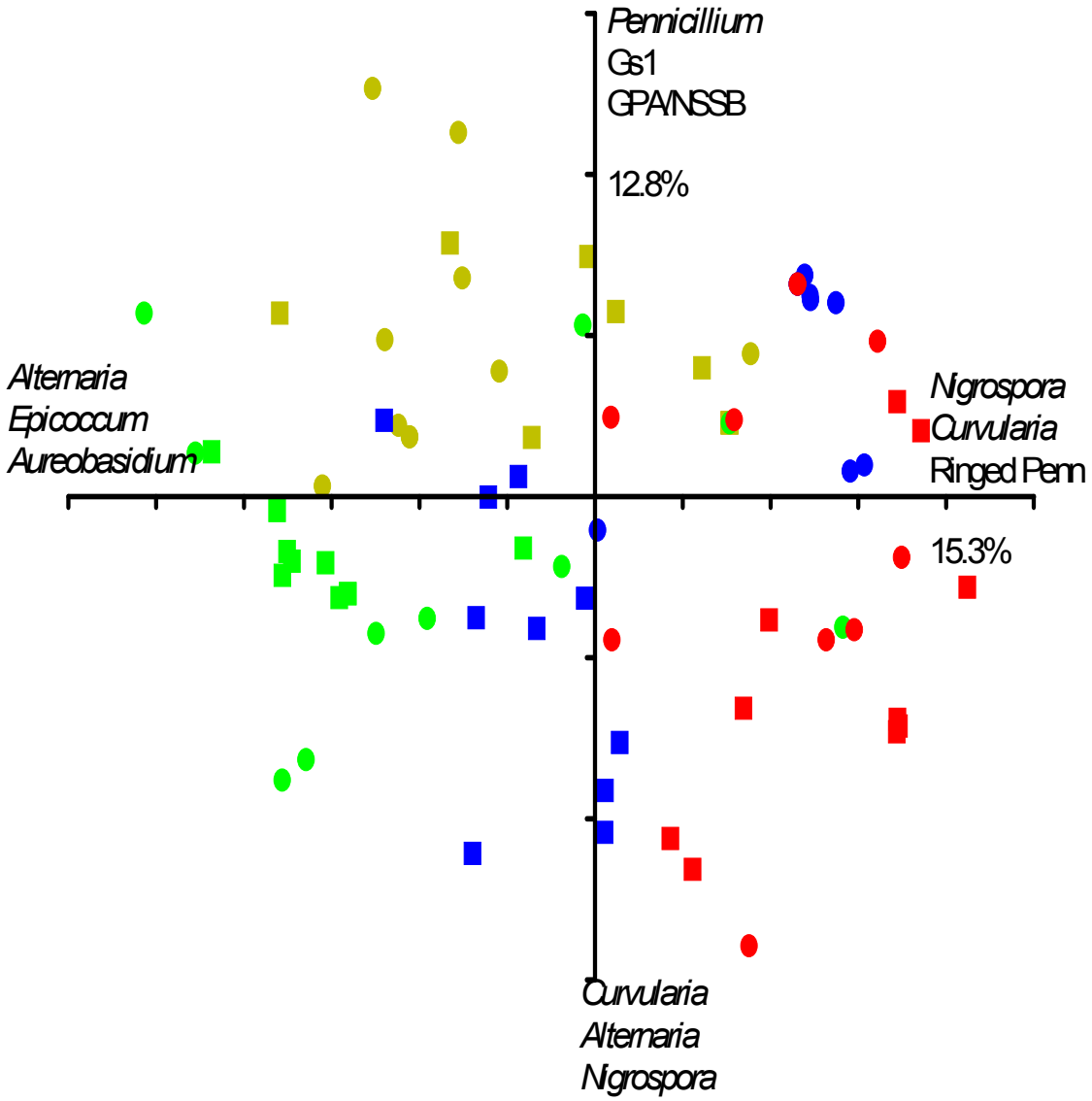

Figure 1. Principle component analysis depicting fungal communities for wild and cultivated 2006 treatments. Squares represent cultivated sites, circles represent wild sites. Yellow points represent April, June is green, August is blue, and October is red. The species that contribute most to the separation along each axis are listed at the ends of that axis. Percentage of variance each axis is responsible for is labeled at the end of that axis. Some data points may occlude other points.

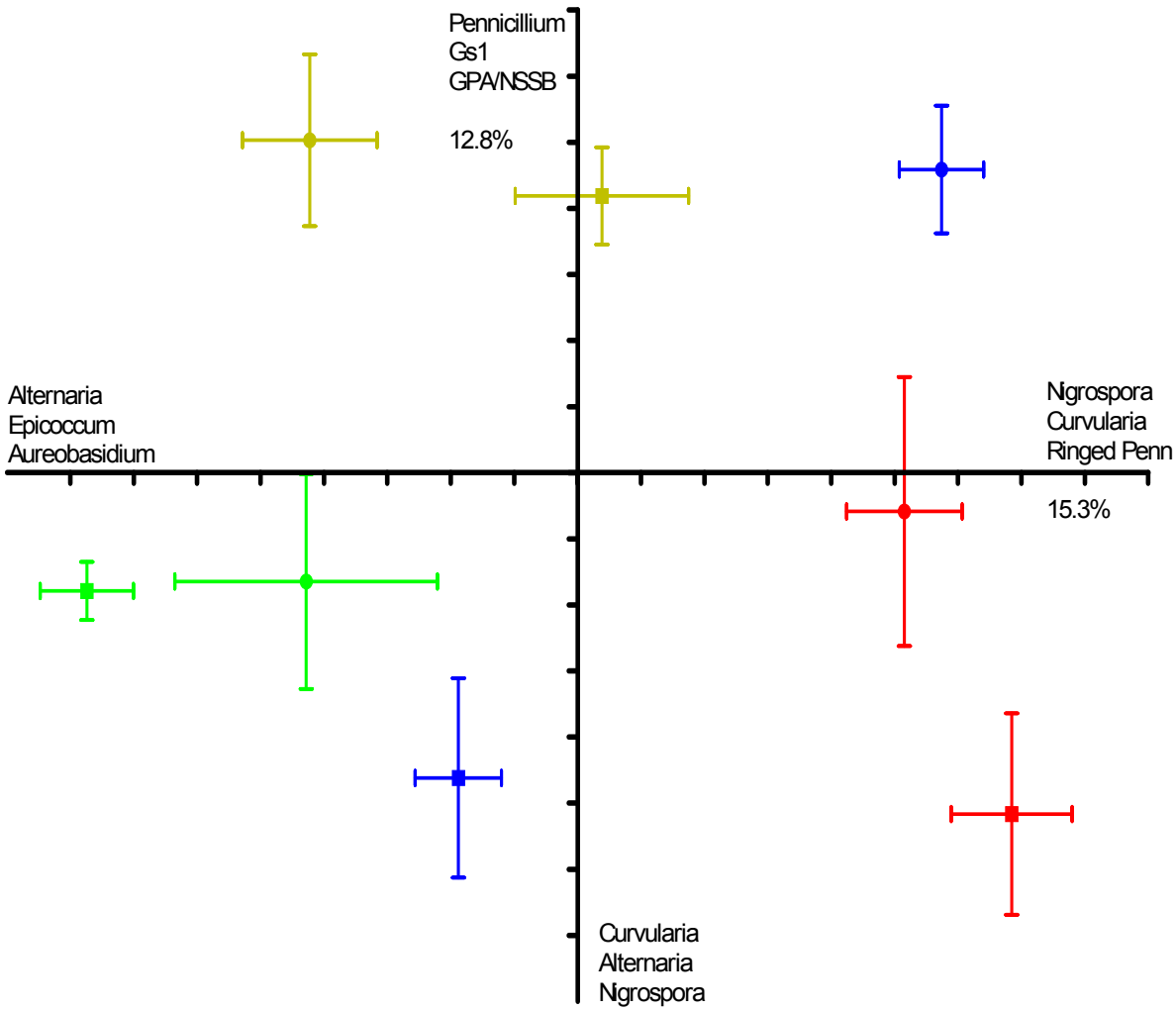

Figure 1a. Principle component analysis depicting fungal communities for wild and cultivated 2006 treatments. Site and date means (+/- SE) are depicted; squares represent cultivated sites, circles represent wild sites. Yellow points represent April, June is green, August is blue, and October is red. The species that contribute most to the separation along each axis are listed at the ends of that axis. Percentage of variance each axis is responsible for is labeled at the end of that axis. Some data points may occlude other points

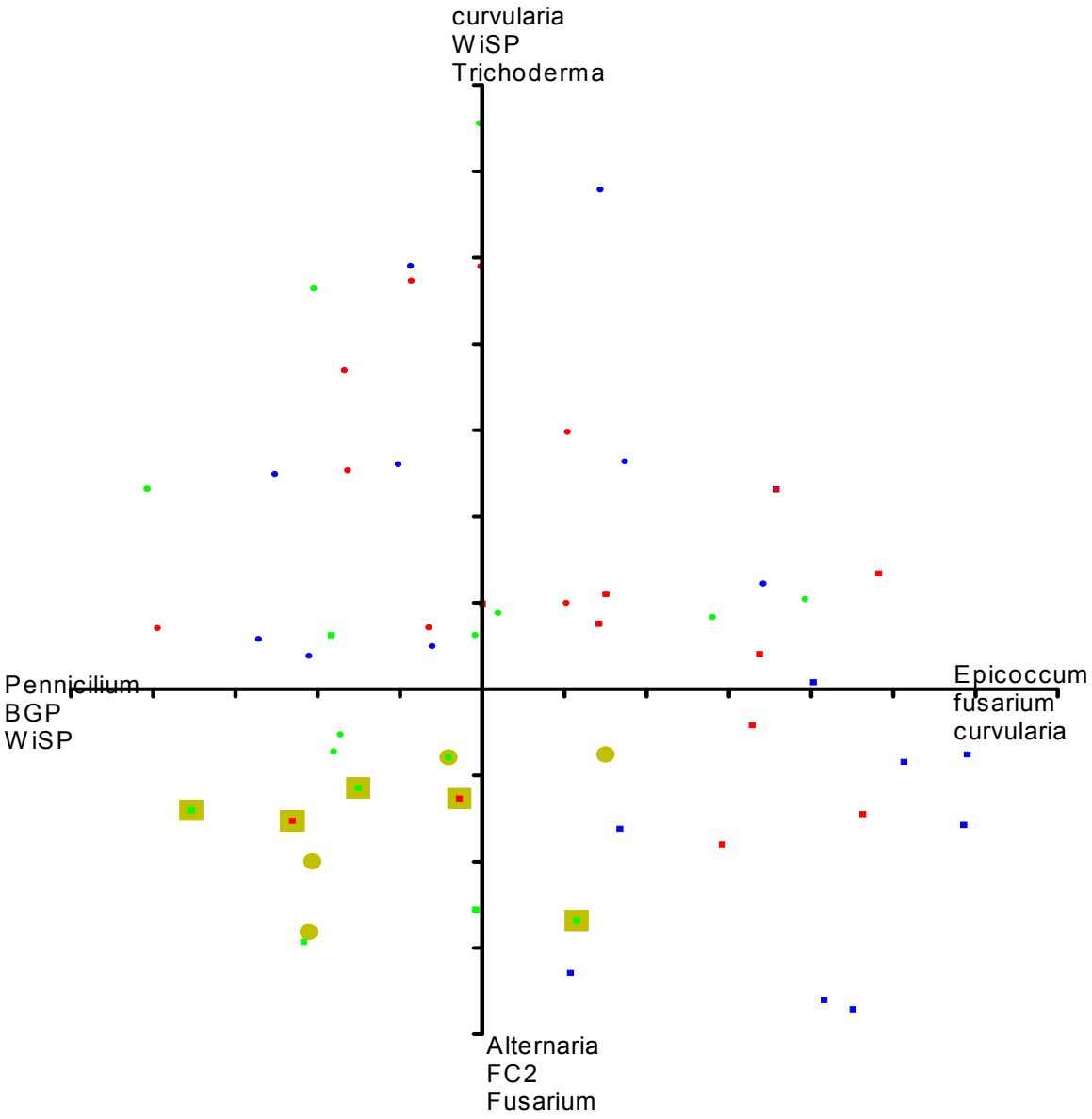

Figure 2. Principle component analysis depicting fungal communities for wild and cultivated 2007 treatments. Squares represent cultivated sites, circles represent wild sites. Yellow points represent April, June is green, August is blue, and October is red. The species that contribute most to the separation along each axis are listed at the ends of that axis. Percentage of variance each axis is responsible for is labeled at the end of that axis. Some data points may occlude other points.

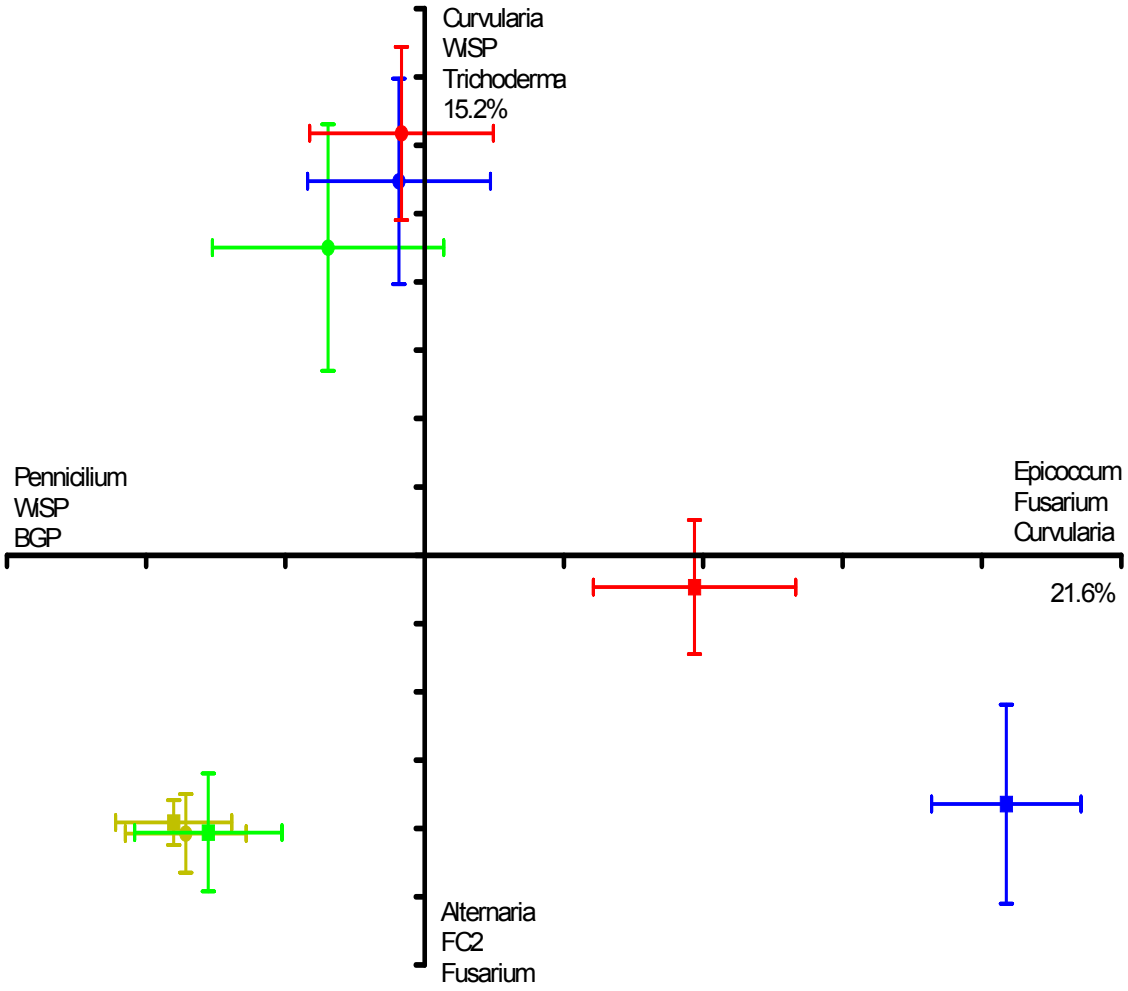

Figure 2a. Principle component analysis depicting fungal communities for wild and cultivated 2007 treatments. Site and date means (+/- SE) are depicted; squares represent cultivated sites, circles represent wild sites. Yellow points represent April, June is green, August is blue, and October is red. The species that contribute most to the separation along each axis are listed at the ends of that axis. Percentage of variance each axis is responsible for is labeled at the end of that axis.

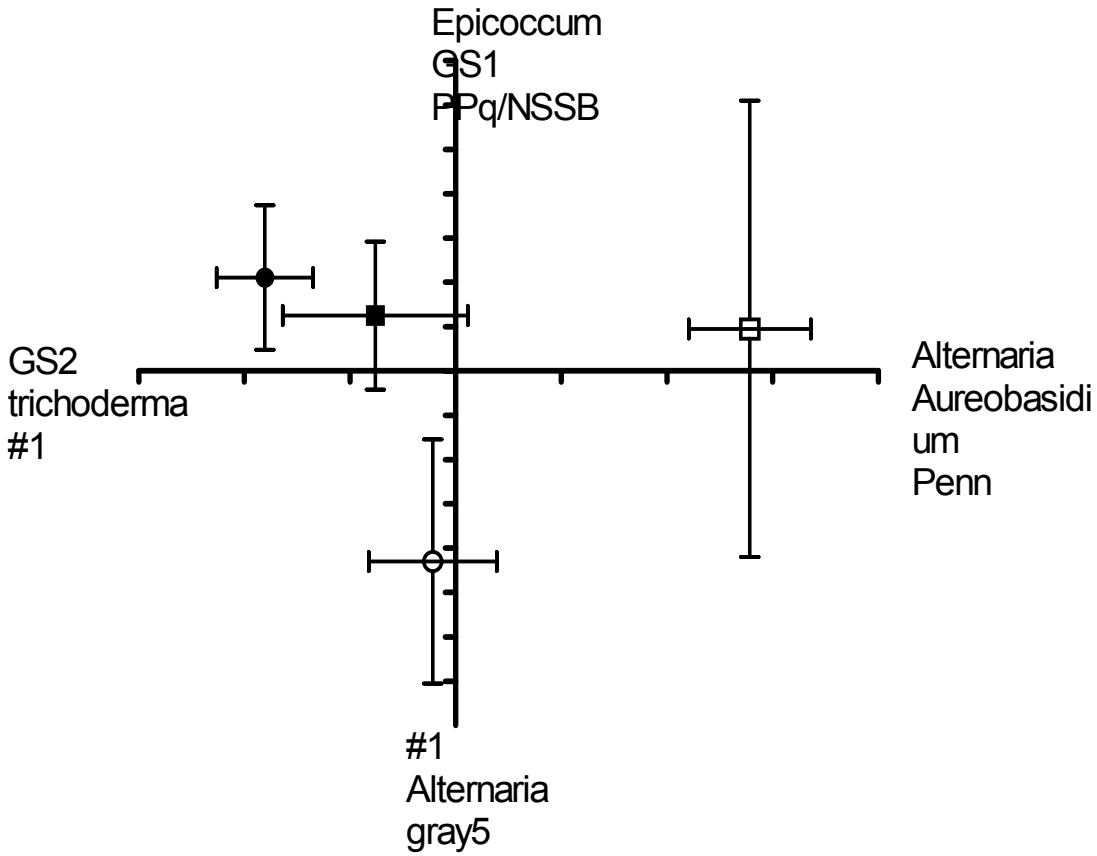

Figure 3. April 2006 & 2007. Principle component analysis depicting fungal communities for wild and cultivated April 2006 and 2007 treatments. Site and date means (+/- SE) are depicted with solid circle representing wild 06; empty circle, wild 2007; solid square, cultivated 2006; empty square, cultivated 2007. The species that contribute most to the separation along each axis are listed at the ends of that axis. Percentage of variance each axis is responsible for is labeled at the end of that axis.

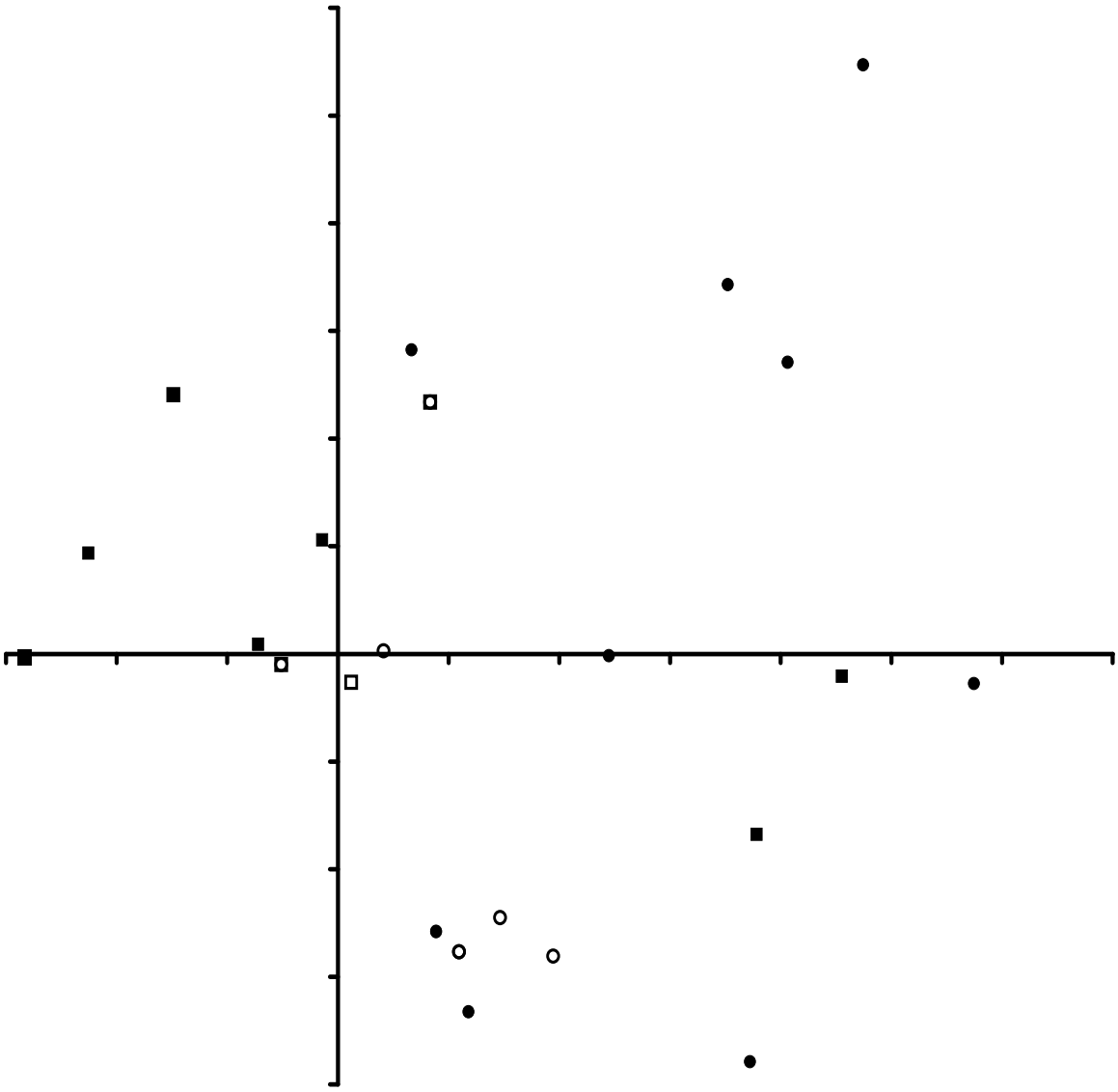

Figure 3a. Principle component analysis with April's individual data points for each location and year. Solid circle, wild 2006, open circle, wild 2007, solid square, cultivated 2006, open square, cultivated 2007. The species that contribute most to the separation along each axis are listed at the ends of that axis. Percentage of variance each axis is responsible for is labeled at the end of that axis. Some data points may occlude other points.

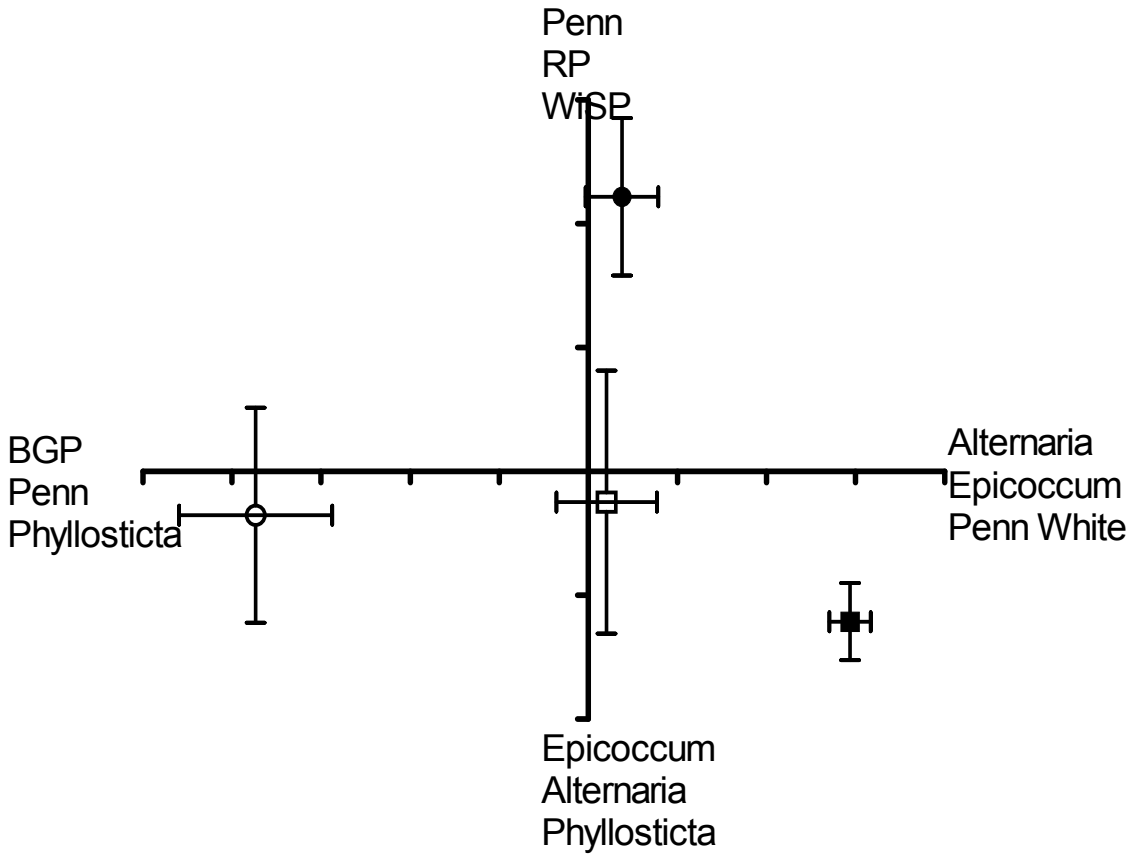

Figure 4. June 2006 & 2007. Principle component analysis depicting fungal communities for wild and cultivated June 2006 and 2007 treatments. Site and date means (+/- SE) are depicted with solid circle representing wild 06; empty circle, wild 2007; solid square, cultivated 2006; empty square, cultivated 2007. The species that contribute most to the separation along each axis are listed at the ends of that axis. Percentage of variance each axis is responsible for is labeled at the end of that axis.

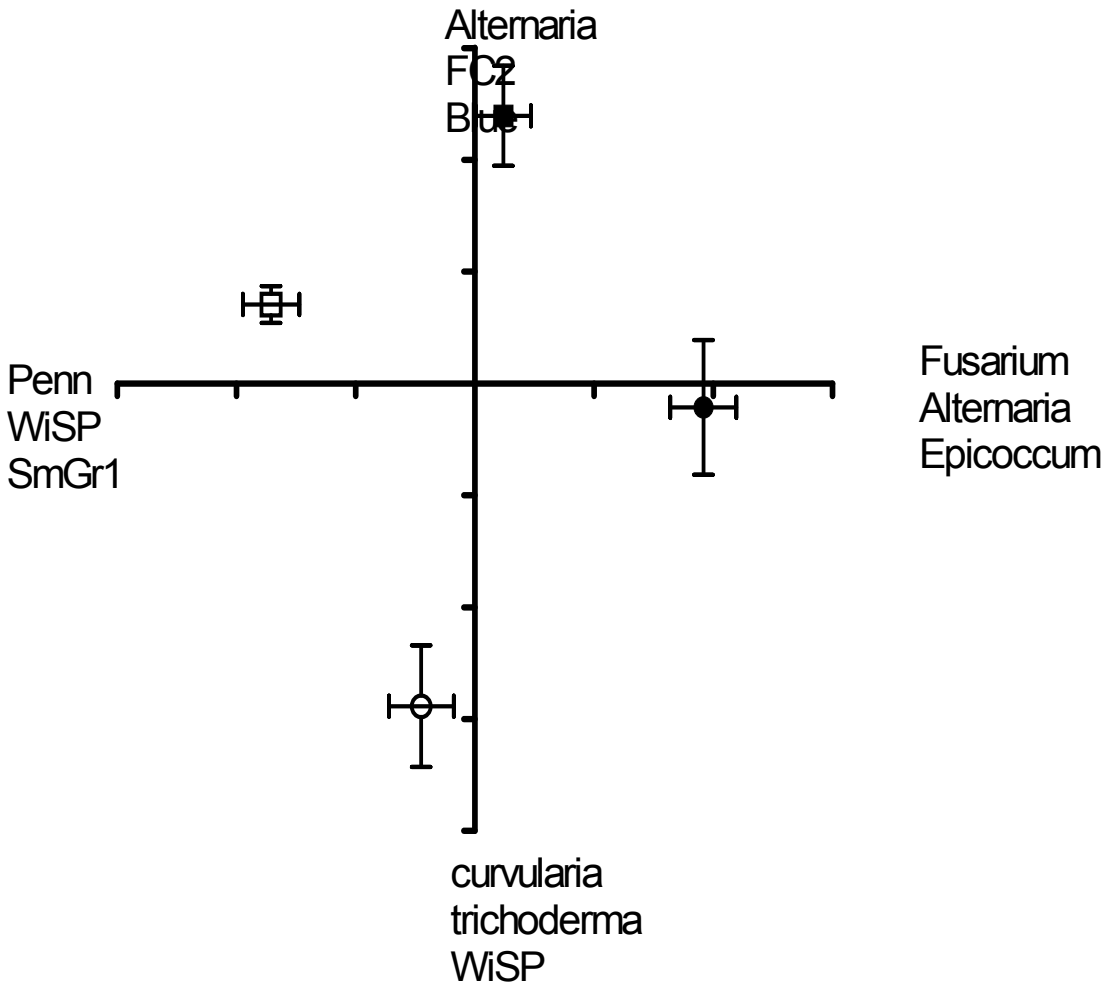

Figure 5. August 2006 & 2007. Principle component analysis depicting fungal communities for wild and cultivated August 2006 and 2007 treatments. Site and date means (+/- SE) are depicted with solid circle representing wild 06; empty circle, wild 2007; solid square, cultivated 2006; empty square, cultivated 2007. The species that contribute most to the separation along each axis are listed at the ends of that axis. Percentage of variance each axis is responsible for is labeled at the end of that axis.

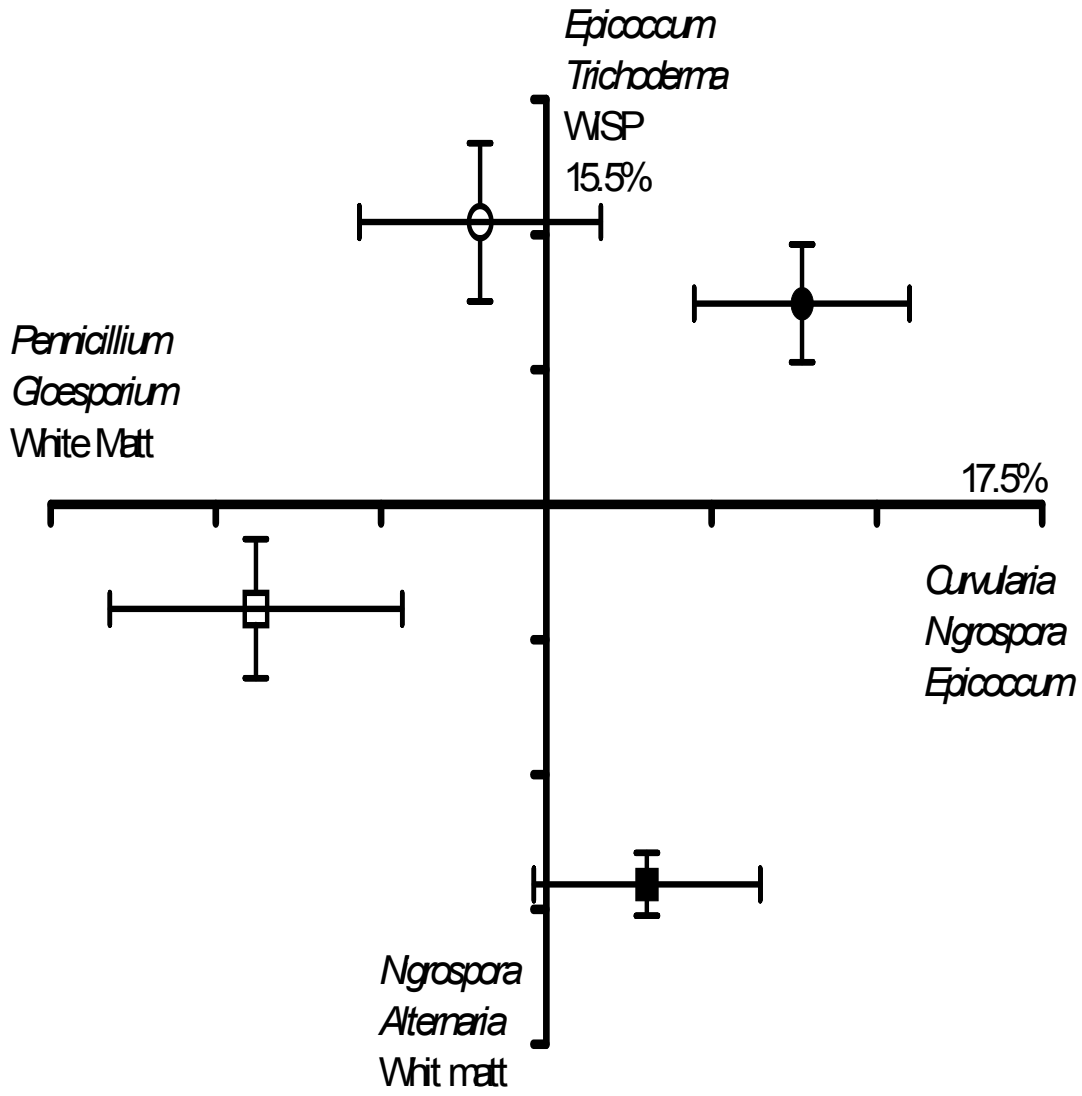

Figure 6. October 2006 & 2007. Principle component analysis depicting fungal communities for wild and cultivated October 2006 and 2007 treatments. Site and date means (+/- SE) are depicted with solid circle representing wild 06; empty circle, wild 2007; solid square, cultivated 2006; empty square, cultivated 2007. The species that contribute most to the separation along each axis are listed at the ends of that axis. Percentage of variance each axis is responsible for is labeled at the end of that axis.

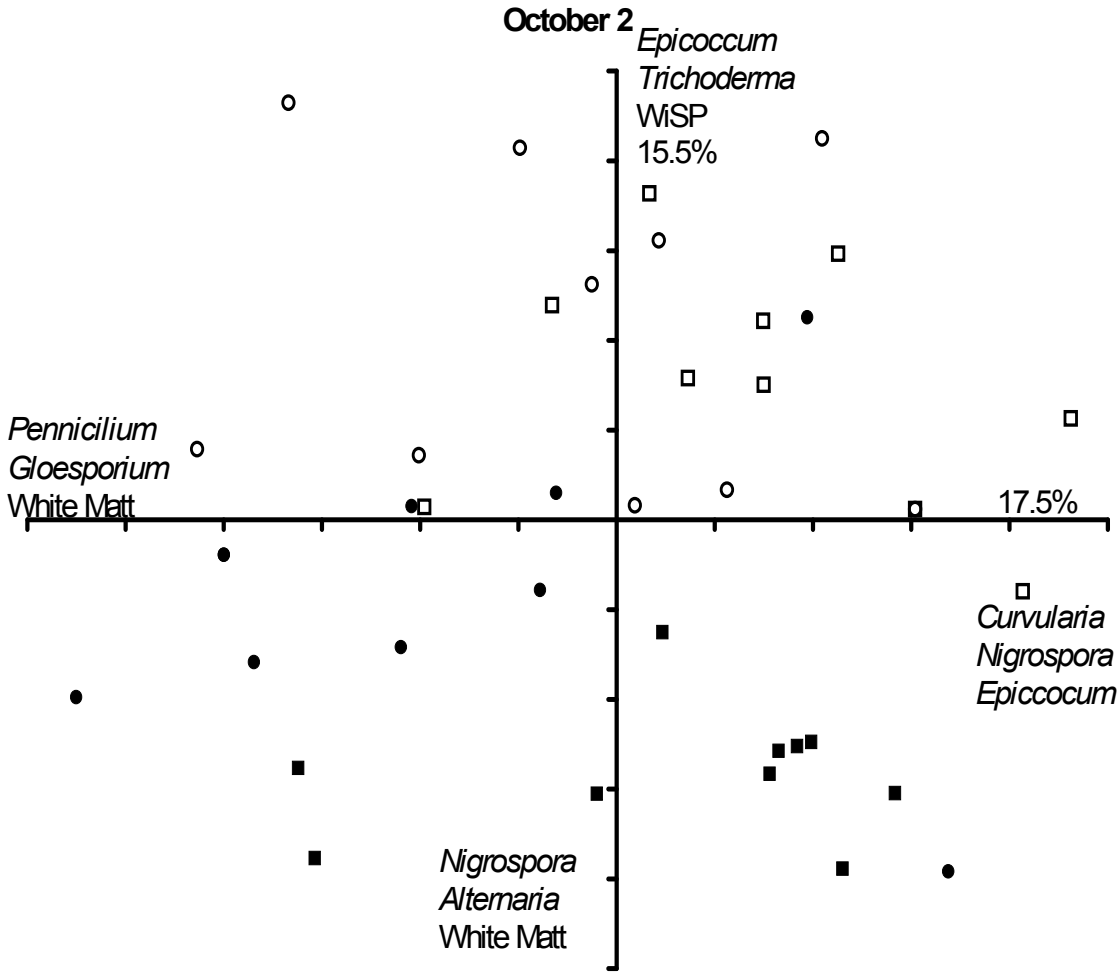

Figure 6a. Principle component analysis with October's individual data points for each location and year. Solid circle, wild 2006, open circle, wild 2007, solid square, cultivated 2006, open square, cultivated 2007. The species that contribute most to the separation along each axis are listed at the ends of that axis. Percentage of variance each axis is responsible for is labeled at the end of that axis. Some data points may occlude other points.

Appendix 1 Traffic volume data

	Counts relevant to Mile Post 25		Counts relevant to Mile Post 35	
	2006	**2007**	**2006**	**2007**
January	1,273,176	1,255,348	1,209,517	1,192,581
February	1,188,559	1,138,151	1,129,131	1,081,243
March	1,437,758	1,390,129	1,365,870	1,320,623
April	1,494,896	1,431,745	1,420,151	1,360,158
May	1,706,987	1,715,520	1,621,638	1,629,744
June	1,877,407	1,957,192	1,783,537	1,859,332
July	2,255,550	2,241,525	2,142,773	2,129,449
August	2,253,279	2,236,990	2,140,615	2,125,141
September	1,643,524	1,708,113	1,561,348	1,622,707
October	1,439,387	1,418,818	1,367,418	1,347,877
November	1,354,309	1,254,294	1,286,594	1,191,579
December	1,329,397	1,215,692	1,262,927	1,154,907
Annual total	19,254,229	18,963,517	18,291,518	18,015,341

Appendix 2 Study sites

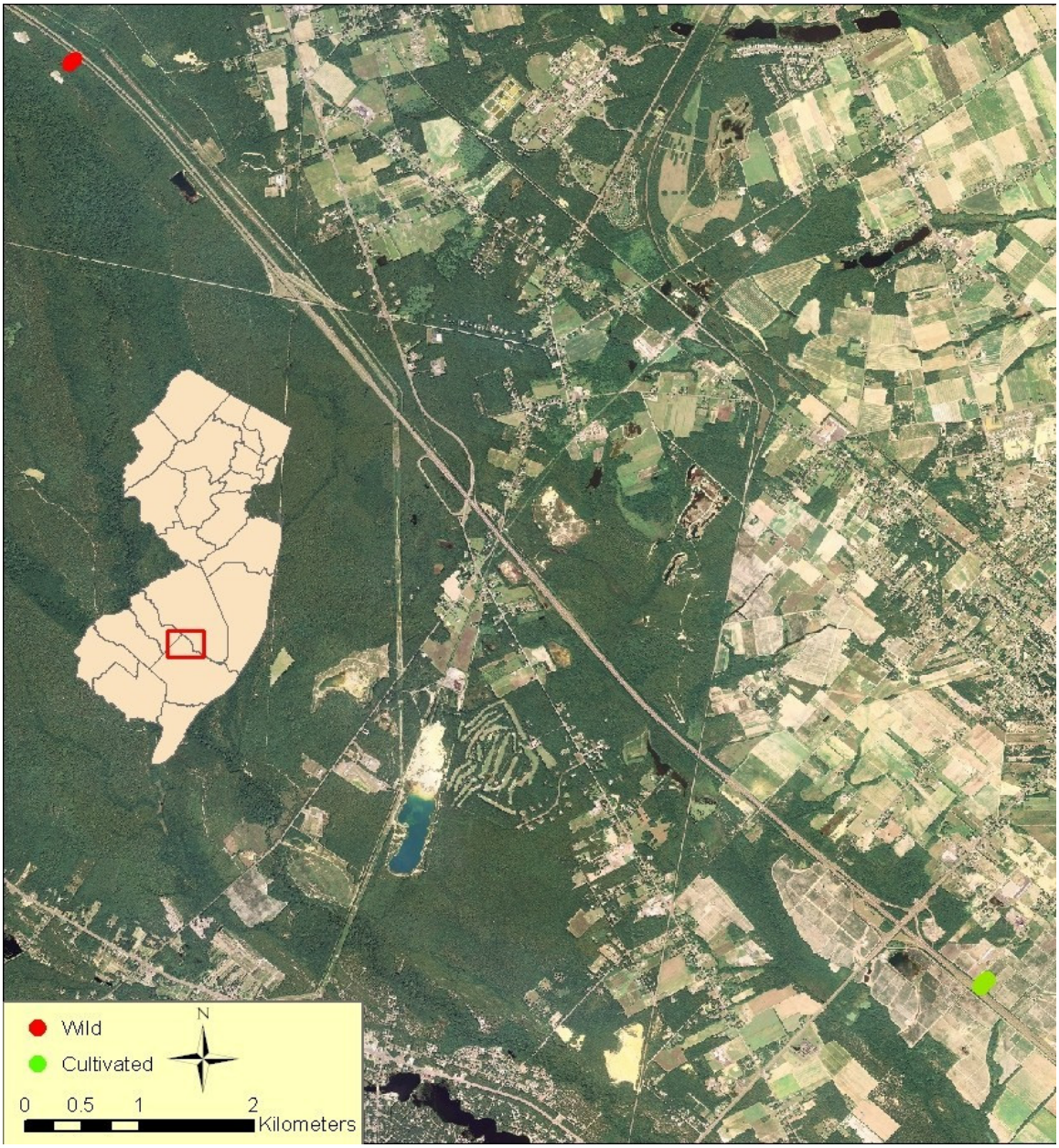

Figure 7. Aerial Photograph of study area, ACE running from the Southeast to the Northwest.

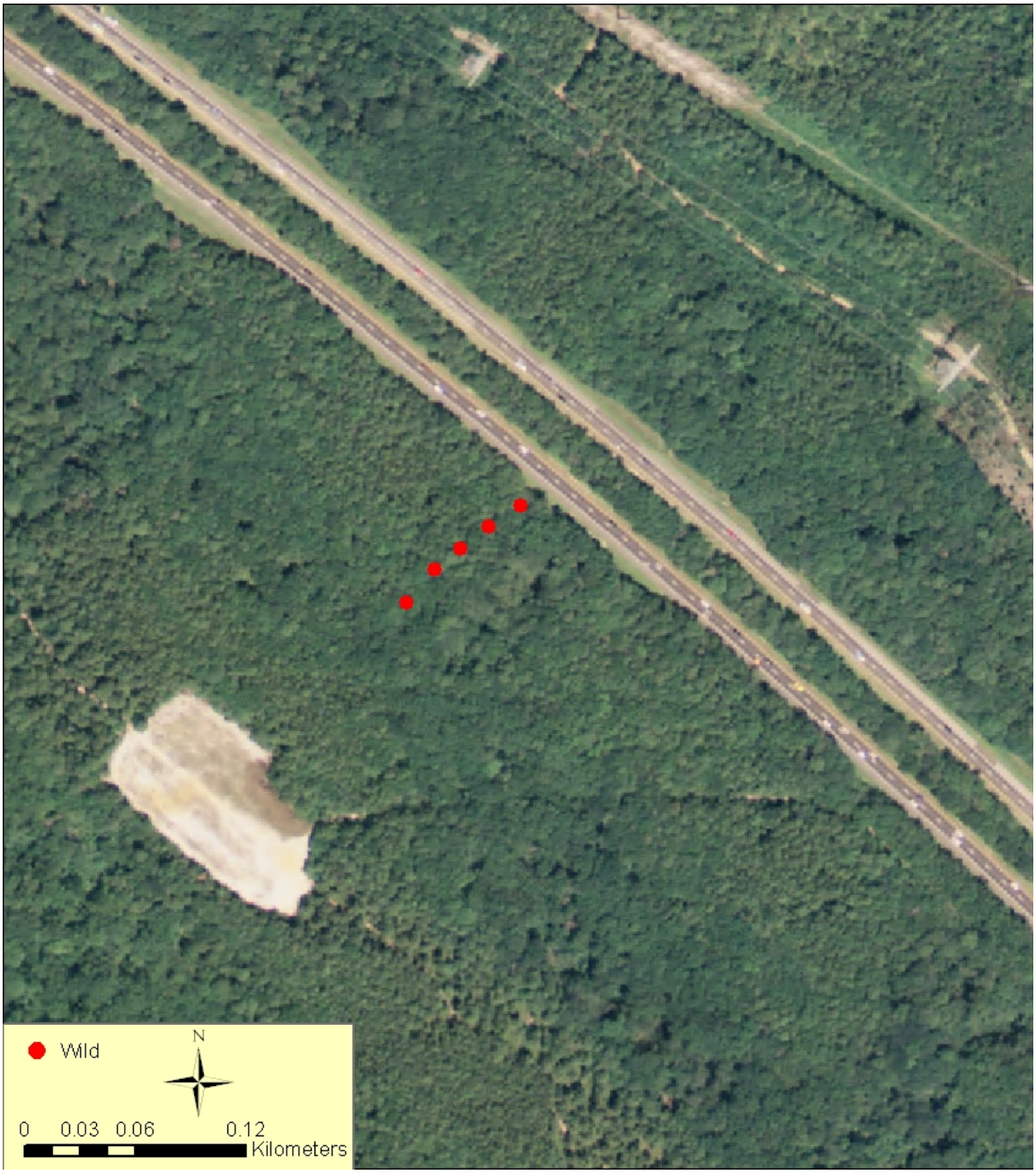

Figure 8. Aerial Photograph of study area, wild site, near mile marker 34.5 on the ACE.

Figure 9. Aerial Photograph of study area, cultivated site, near mile marker 27.3 on the ACE.

Appendix 3 Fungicide and pesticide applications

2007 and 2008 spray records			
Date	Substance	Application Rate	Classification
2007			
March 27	Lime Sulfer	4 gallons / acre	Fungicide
April 30	Zyram 70 ®	4 lbs. / acre	Fungicide
May 9	Pristine ®	20 oz. / acre	Fungicide
May 19	Dipol ®	1 lb. / acre	Insecticide
May 19	Zyram ®	4 lbs. / acre	Fungicide
May 27	Guthion 50W ®	1 lb / acre	Insecticide
May 27	Captec ®	2 quarts / acre	Fungicide
June 7	Lanate ®	1 lb. / acre	Insecticide
June 7	Captec ®	2 quarts / acre	Fungicide
June 23	Provado 1.6 ®	4 oz. / acre	Fungicide
June 23	Imidan 70W ®	1-1/3 lbs. / acre	Insecticide
June 24	Provado ®	4 oz / acre	Fungicide
June 24	Imidan 70W ®	1-1/3 lbs. / acre	Insecticide
June 25	Captec ®	2 quarts / acre	Fungicide
July 10	Danitol ®	10.7 oz. / acre	Insecticide
September 13	Asana ®	8 oz. / acre	Insecticide
2008			
April 3	Indar 2F ®	6 oz. / acre	Fungicide
April 11	Indar 2F ®	6 oz. / acre	Fungicide
April 23	Abound ®	10 oz. / acre	Fungicide
May 5	Zyram ®	4 lbs. / acre	Fungicide
May 5	Pristine ®	8 oz. / acre	Fungicide
May 5	Crymax ®	1 lb. / acre	Insecticide
May 22	Guthion 50W ®	1 lb. / acre	Insecticide
May 22	Captec ®	2 quarts / acre	Fungicide
June 2	Imidan 70 W ®	1-1/3 lb. / acre	Insecticide
June 2	Provado 1.6 ®	4 oz. / acre	Fungicide
June 19	Captec ®	2 quarts / acre	Fungicide
June 19	Assail ®	4 oz. / acre	Insecticide
June 29	Assail ®	4 oz. / acre	Insecticide
July 7	Danitol ®	10.7 oz. / acre	Insecticide
September 2	Malathion ®	1 ½ pints / acre	Insecticide

Appendix 4 Meteorological data

	Precipitation mm		Relative Humidity %		Mean Air Temp ºC	
	2006	**2007**	**2006**	**2007**	**2006**	**2007**
April	80.26	148.8	60.75	63.92	11.52	8.86
May	153.94	165.17	63.3	61.19	15.81	16.61
June	113.54	81.03	74.39	68.01	20.62	20.97
July	114.81	135.39	72.93	69.89	24.41	22.79
August	75.95	92.96	71.14	75.44	23.31	22.45
September	261.61	111.76	76.43	71.86	18.09	19.64
October	118.87	106.68	68.64	78.09	12.52	16.42

	Solar Radiation Watts / M square		Net Radiation Watts / M square	
	2006	**2007**	**2006**	**2007**
April	205.81	167.42	119.06	97.45
May	235.17	251.66	84.32	25.15
June	199.78	222.82	132.84	156.04
July	222.26	225.79	156.1	157.14
August	203.85	186.81	137.81	127.68
September	153.54	188.38	88.81	117.15
October	120.45	116.96	52.23	59.99

Appendix 5 ANOVA results

SPECIES RICHNESS

Dependent Variable: Species Richness Cultivated 2006 Communities

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	80.60	4.240	4.99	0.0004
Error	20	17.00	0.850		
Corrected Total	39	97.600			

R-Square	Coeff Var	Root MSE	sr Mean
0.825820	29.74047	0.921954	3.100000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
month	3	49.400	16.4667	19.37	<.0001
pos	4	7.850	1.9625	2.31	0.0934
month*pos	12	23.350	1.9458	2.29	0.0490

Dependent Variable: Species Richness Wild 2006 Communities

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	108.4750	5.7092	1.64	0.1395
Error	20	69.5000	3.4750		
Corrected Total	39	177.9750			

R-Square	Coeff Var	Root MSE	sr Mean
0.609496	53.64418	1.864135	3.475000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Month	3	27.6750	9.2250	2.65	0.0764
pos	4	16.8500	4.2125	1.21	0.3367
month*pos	12	63.9500	5.3292	1.53	0.1922

Dependent Variable: Species Richness Cultivated 2007 Communities

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	105.1000	5.5316	2.05	0.0599
Error	20	54.0000	2.7000		
Corrected Total	39	159.1000			

R-Square	Coeff Var	Root MSE	sr Mean
0.660591	62.00633	1.643168	2.650000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
month	3	82.9000	27.6333	10.23	0.0003
pos	4	5.3500	1.3375	0.50	0.7393
month*pos	12	16.8500	1.4042	0.52	0.8771

Dependent Variable: Species Richness Wild 2007 Communities

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	104.1000	5.4789	2.07	0.0577
Error	20	53.0000	2.6500		
Corrected Total	39	157.1000			

R-Square	Coeff Var	Root MSE	sr Mean
0.662635	48.59349	1.627882	3.350000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
month	3	67.5000	22.5000	8.49	0.0008
pos	4	9.1000	2.2750	0.86	0.5055
month*pos	12	27.5000	2.2917	0.86	0.5916

Dependent Variable: Species Richness cultivated 2006 and 2007

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	189.750	4.8654	2.74	0.0010
Error	40	71.000	1.7750		
Corrected Total	79	260.750			

R-Square	Coeff Var	Root MSE	sr Mean
0.727709	46.34057	1.332291	2.875000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
yr	1	4.050	4.050	2.28	0.1388
pos	4	4.625	1.156	0.65	0.6293
yr*pos	4	8.575	2.144	1.21	0.3226
mo	3	128.250	42.750	24.08	<.0001
yr*mo	3	4.050	1.350	0.76	0.5229
pos*mo	12	25.875	2.156	1.21	0.3070
yr*pos*mo	12	14.325	1.194	0.67	0.7667

Dependent Variable: Species Richness wild 2006 and 2007

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	212.888	5.4587	1.78	0.0363
Error	40	122.500	3.063		
Corrected Total	79	335.388			

R-Square	Coeff Var	Root MSE	sr Mean
0.634751	51.28205	1.750000	3.412500

Source	DF	Anova SS	Mean Square	F Value	Pr > F
yr	1	0.3125	0.3125	0.10	0.7511
pos	4	16.8250	4.2062	1.37	0.2604
yr*pos	4	9.1250	2.2813	0.74	0.5672
mo	3	60.0375	20.0125	6.53	0.0011
yr*mo	3	35.1375	11.7125	3.82	0.0169
pos*mo	12	37.2750	3.1063	1.01	0.4545
yr*pos*mo	12	54.1750	4.5146	1.47	0.1746

Dependent Variable-Species Richness cultivated April Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	27.450	3.050	8.71	0.0011
Error	10	3.500	0.350		
Corrected Total	19	30.950			

R-Square	Coeff Var	Root MSE	sr Mean
0.886914	62.27452	0.591608	0.950000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
yr	1	2.450	2.45	7.00	0.0245
pos	4	22.20	5.55	15.86	0.0002
yr*pos	4	2.80	0.70	2.00	0.1705

Dependent Variable-Species Richness wild April samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	57.800	6.4222	2.92	0.0552
Error	10	22.000	2.2000		
Corrected Total	19	79.800			

R-Square	Coeff Var	Root MSE	sr Mean
0.724311	51.14620	1.483240	2.900000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
yr	1	28.800	28.800	13.09	0.0047
pos	4	12.800	3.200	1.45	0.2867
yr*pos	4	16.200	4.050	1.84	0.1977

Dependent Variable-Species Richness cultivated June samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	10.800	1.200	1.20	0.3878
Error	10	10.000	1.00		
Corrected Total	19	20.800			

R-Square	Coeff Var	Root MSE	sr Mean
0.519231	38.46154	1.000000	2.600000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
yr	1	5.00	5.00	5.00	0.0493
pos	4	2.30	0.58	0.58	0.6873
yr*pos	4	3.50	0.88	0.88	0.5121

2006 PCA Dependent Variable: pc1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	25.0586	0.6425	5.17	<.0001
Error	40	4.975	0.12437		
Corrected Total	79	30.034			

R-Square	Coeff Var	Root MSE	pc1 Mean
0.834350	28213716	0.352671	1.25E-6

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	0.2857	0.2857	2.30	0.1375
month	3	15.9033	5.3011	42.62	<.0001
site*month	3	4.4209	1.4736	11.85	<.0001
pos	4	0.5881	0.1470	1.18	0.3334
site*pos	4	0.1217	0.0304	0.24	0.9112
month*pos	12	1.6043	0.1337	1.07	0.4057
site*month*pos	12	2.1346	0.1779	1.43	0.1928

2006 PCA Dependent Variable: pc2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	18.5627	0.47597	2.91	0.0005
Error	40	6.5326	0.16331		
Corrected Total	79	25.0952			

R-Square	Coeff Var	Root MSE	pc2 Mean
0.739689	8082429	0.404121	5E-6

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	2.7277	2.7277	16.70	0.0002
month	3	6.4921	2.1640	13.25	<.0001
site*month	3	2.5947	0.8649	5.30	0.0036
pos	4	1.4810	0.3703	2.27	0.0789
site*pos	4	0.1810	0.0453	0.28	0.8911
month*pos	12	1.6467	0.1372	0.84	0.6102
site*month*pos	12	3.4395	0.2866	1.76	0.0908

2007 PCA Dependent Variable: pc1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	25.765	0.660	2.60	0.0016
Error	40	10.148	0.254		
Corrected Total	79	35.913			

R-Square	Coeff Var	Root MSE	pc1 Mean
0.717419	-40295788	0.503697	-0.000001

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	2.3782	2.3782	9.37	0.0039
month	3	11.4033	3.8010	14.98	<.0001
site*month	3	5.1786	1.7262	6.80	0.0008
pos	4	1.7894	0.4473	1.76	0.1553
site*pos	4	0.7608	0.1902	0.75	0.5641
month*pos	12	2.8174	0.2348	0.93	0.5316
site*month*pos	12	1.4373	0.1198	0.47	0.9193

2007 PCA Dependent Variable: pc2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	19.688	0.505	3.63	<.0001
Error	40	5.561	0.139		
Corrected Total	79	25.249			

R-Square	Coeff Var	Root MSE	pc2 Mean
0.779737	14915087	0.372877	2.5E-6

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	7.2928	7.2928	52.45	<.0001
month	3	4.9945	1.6648	11.97	<.0001
site*month	3	2.7298	0.9099	6.54	0.0010
pos	4	0.1277	0.0319	0.23	0.9201
site*pos	4	0.0646	0.0162	0.12	0.9760
month*pos	12	3.0043	0.2504	1.80	0.0815
site*month*pos	12	1.4741	0.1228	0.88	0.5698

April PCA Dependent Variable: pc1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	7.277	2.4257	9.25	0.0001
Error	36	9.443	0.2623		
Corrected Total	39	16.720			

R-Square	Coeff Var	Root MSE	pc1 Mean
0.435223	3414425	0.512164	0.000015

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	4.119	4.119	15.70	0.0003
yr	1	2.559	2.559	9.75	0.0035
site*yr	1	0.600	0.600	2.29	0.1393

April PCA Dependent Variable: pc2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.635	0.211	0.86	0.4727
Error	36	8.894	0.247		
Corrected Total	39	9.529			

R-Square	Coeff Var	Root MSE	pc2 Mean
0.066576	6627453	0.497059	7.5E-6

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	0.281	0.281	1.14	0.2932
yr	1	0.120	0.121	0.49	0.4893
site*yr	1	0.233	0.233	0.94	0.3382

June PCA Dependent Variable: pc1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	14.306	4.767	22.49	<.0001
Error	36	7.633	0.212		
Corrected Total	39	21.939			

R-Square	Coeff Var	Root MSE	pc1 Mean
0.652080	-4604634	0.460463	-0.000010

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	6.869	6.869	32.40	<.0001
yr	1	7.040	7.040	33.21	<.0001
site*yr	1	0.397	0.396	1.87	0.1800

June PCA Dependent Variable: pc2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4.338	1.446	4.02	0.0145
Error	36	12.948	0.3597		
Corrected Total	39	17.286			

R-Square	Coeff Var	Root MSE	pc2 Mean
0.250915	11994628	0.599731	5E-6

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	0.632	0.632	1.76	0.1933
yr	1	2.175	2.175	6.05	0.0189
site*yr	1	1.530	1.53	4.25	0.0465

August PCA Dependent Variable: pc1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	17.146	5.715	34.96	<.0001
Error	36	5.885	0.163		
Corrected Total	39	23.031			

R-Square	Coeff Var	Root MSE	pc1 Mean
0.744479	16172695	0.404317	2.5E-6

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	11.636	11.636	71.18	<.0001
yr	1	5.401	5.401	33.04	<.0001
site*yr	1	0.101	0.101	0.67	0.4182

August PCA Dependent Variable: pc2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	9.117	3.039	21.95	<.0001
Error	36	4.984	0.138		
Corrected Total	39	14.101			

R-Square	Coeff Var	Root MSE	pc2 Mean
0.646526	-14883994	0.372100	-0.000002

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	2.968	2.968	21.44	<.0001
yr	1	5.998	5.998	43.32	<.0001
site*yr	1	0.150	0.151	1.09	0.3033

October PCA Dependent Variable: pc1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3.758	1.2529	3.63	0.0219
Error	36	12.425	0.345		
Corrected Total	39	16.183			

R-Square	Coeff Var	Root MSE	pc1 Mean
0.232199	-5.2918E18	0.587505	-0.000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	2.908	2.908	8.43	0.0063
yr	1	0.8223	0.822	2.38	0.1314
site*yr	1	0.0273	0.027	0.08	0.7803

October PCA Dependent Variable: pc2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	9.449	3.149	23.57	<.0001
Error	36	4.810	0.134		
Corrected Total	39	14.258			

R-Square	Coeff Var	Root MSE	pc2 Mean
0.662641	14621492	0.365537	2.5E-6

Source	DF	Anova SS	Mean Square	F Value	Pr > F
site	1	1.092	1.093	8.18	0.0070
yr	1	8.031	8.031	60.10	<.0001
site*yr	1	0.325	0.325	2.43	0.1277