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Seasonality and management, not proximity to highway, affect species richness and community composition of epiphytic phylloplane fungi found on (wild and cultivated) *Vaccinium* spp.

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ARTICLE INFO

Article history:
Received 25 September 2010
Revision received 2 January 2011
Accepted 16 February 2011
Available online 10 May 2011
Corresponding editor:
Kevin Hyde

Keywords:
Agriculture
Automobile traffic
Blueberry
Highway
Leaf surface
Phenology
Phylloplane fungi
Principle component analysis

ABSTRACT

The phylloplane is a cryptic environment that harbors a variety of pathogens and pathogen antagonists, and these populations are affected by many factors such as weather, season, plant location and leaf phenology. To address the hypothesis that pollution from a major highway would influence phylloplane communities, blueberry leaves were collected in Apr., Jun., Aug., and Oct. over 2 years from bushes in wild areas and cultivated farms along transects perpendicular to the Atlantic City Expressway. Community structure and species richness changed monthly, annually, and from site to site. 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 fungal communities. Leaves collected in Apr. had significantly lower species richness than those collected 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.

Introduction

The leaf surface (phylloplane) is an important substratum for microbial life (Lindow & Brandl 2003) that has been suggested to be an "ecologically neglected milieu" (Fokkema 1991). The phyllosphere is considered a hostile environment in that it can experience daily and seasonal changes in water availability, incident irradiation and nutrient availability (Hirano & Upper 2000; Lindow & Leveau 2002). Seasonal changes alter leaf morphology, and leaf surface biochemistry has been seen to affect the community composition and magnitude of phylloplane microbial populations (Hirano & Upper 2000; Yadav *et al.* 2005). As a leaf ages, the surface texture becomes increasingly rough (Mechaber *et al.* 1996), possibly as a result of microbial action (Knoll & Schreiber 2000), thus altering the substratum to provide a unique island resource for fungal colonization. Also, the effects of grazing fauna (aphids and other insects) can influence leaf surface resources

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doi:10.1016/j.funeco.2011.02.003

(Stadler & Michalzik 1999). Within this fungal community, changes in resources and competition between organisms dictate the actual community composition (Kinkel 1997; Lindow & Leveau 2002; Lindow & Brandl 2003), leading to specific fungal communities (Osono *et al.* 2004; Osono & Mori 2004) and microbial processes (Ruinen 1970).

Phyllosphere microbial communities provide novel strategies for disease management, including biological control, habitat alteration and the modification of plant traits to interfere with foliar pathogens (Lindow & Leveau 2002; Morris & Kinkel 2002; Stohr & Dighton 2004; Kaewchai *et al.* 2009).

The surrounding environment, including local flora, influences atmospheric microbial propagule density and composition at a given location geographically (Bovallius *et al.* 1978) and on trees (Jurkevitch & Shapira 2000; Osono & Mori 2004). This environment dictates what types and numbers of microbes settle and grow on the phylloplane (Lindemann & Upper 1985). Position on the tree itself influences microbial populations with lower locations on the tree experiencing less variation in abiotic factors, creating more stable environments for microbes (Kinkel 1997). Other studies indicate that shoot age may influence phylloplane communities (Osono & Mori 2004).

Environmental acidifying pollutants and ozone have also been shown to affect phylloplane fungal communities (Robinson 1971). The presence of zinc, lead and cadmium reduced phyllosphere microbial abundance and diversity of cabbages and pine saplings when compared with their non-contaminated counterparts (Gingell *et al.* 2003). Heavy metal and carbon monoxide exposure associated with heavily trafficked roads in Moscow, Russia influenced the population of microorganisms in soil and surface air (Kul'ko & Marefenina 2001). Other studies in India found that "the distribution pattern of phylloplane fungi in polluted sites was markedly affected by air pollutants being emitted from automobiles" (Niwas *et al.* 1988). The study also stated that "The distribution pattern of phylloplane fungi on *Cassia fistula* and *Thevetia neriifolia* plants in air polluted zones gave interesting information on the tolerance of automobile exhaust pollutants by some fungi, which could lead to fungi being good indicators of air pollution" (Niwas *et al.* 1988).

Cultivated crops are influenced by management practices, such as the application of pesticides and fungicides, that further alter phylloplane communities (de Jager *et al.* 2001). Studies in New Zealand found organic apple farms had higher numbers and species richness of phylloplane microbes than managed sites (Waipara *et al.* 2002).

The objective of the present study was to compare fungal phylloplane diversity on wild and cultivated highbush blueberries (*Vaccinium corymbosum*) along a transect perpendicular to the Atlantic City Expressway, NJ, USA over two growing seasons to determine:

(1) How do phyllosphere fungal community composition and species richness vary among wild and cultivated individuals of *V. corymbosum*? Studies by Waipara *et al.* (2002) found differences in phylloplane community composition in cultivated apple orchards under different management techniques. Thus, a difference between managed and wild plants was anticipated. Studies by Bovallius *et al.* (1978) noted that surrounding vegetation affects airborne microbial propagule density and composition, thus influencing phyllosphere microbe populations.

(2) How does seasonal and climate variability over 2 years affect phyllosphere fungal community composition? Seasonal changes in the biophysical properties of leaf surfaces occur and fungal phylloplane community structure also changes as the growing season progresses. Bakker *et al.* (2002) demonstrated seasonal changes in phylloplane community density and structure of apple trees.

(3) How does distance from a source of pollutants derived from motor vehicles affect richness and community composition of phylloplane fungal species? Kul'ko & Marefenina (2001) and Niwas *et al.* (1988) noted an influence of automotive exhaust on phylloplane communities. 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 (*Vaccinium* spp.) are lacking.

Materials and methods

Study sites

Study sites were in the Pine Barrens of southern New Jersey. This forested area is the largest uninterrupted forest on the eastern coastal plain and encompasses over 1 million acres (22 % of New Jersey) (McCormick & Jones 1973). Upland forests are dominated by pitch pine *Pinus rigida* and a number of overstory oak species (McCormick & Jones 1973) with an understory of these communities composed of ericaceous shrubs, such as huckleberry (*Gaylussacia bacata*, *Gaylussacia frondosa*) and blueberry, *Smilax* spp. and *Clethra alnifolia*. Climate is cool temperate with a Jan. mean of 0.3 °C and Jun. mean of 23.8 °C, and annual precipitation means 1 123 ± 182 mm (State Climatologist of NJ). The area frequently experiences high-intensity wildfires (Forman 1979).

The Atlantic City Expressway was selected as a possible point source of pollution within the forest. The Atlantic City Expressway 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 late May and the beginning of Sep. (South Jersey Transportation Authority), when blueberries are in full leaf.

Forested site with blueberry shrubs in understory

Wild sites were selected in lowland forest, dominated by mixed pines and hardwoods. Understory vegetation included *Smilax* spp., *C. alnifolia* and patches of *Sphagnum* spp. Bushes were sampled along two adjacent transects perpendicular to and running 100 m south of the Atlantic City Expressway. Bushes from five points along each transect were sampled every 20 m. Leaf samples were taken from outer mid level,