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Epidemiology and impacts of a leaf spot disease in *Veratrum viride* (Melanthiaceae)

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ABSTRACT. Fungal phytopathogens can cause disease epidemics in crops, weeds, and populations of native plants. To investigate the impact of a foliar phytopathogen on the native herbaceous species, *Veratrum viride*, a demographic and disease assessment was carried out on two high elevation grassy bald populations on Roan Mountain, Tennessee. A leaf spot disease impacted all plants in both populations, causing widespread premature senescence of leaves and stems. Disease severity increased over the course of the growing season. Based on host disease symptoms and fungal conidia morphology, *Pseudocercosporella sublineolata* was shown to be the causal pathogen. A study of herbarium specimens showed no evidence that the disease was epidemic in the species and no evidence of an increase in disease prevalence over time. However, the disease was more common in the mid Atlantic and southern Appalachian regions, but rare in New England.

Key words: climate change, disease severity, foliar disease, natural population, plant pathogen, disease prevalence, *Pseudocercosporella sublineolata*, Roan Mountain, temporal

Infectious diseases can devastate plantings in genotypically uniform crops cultivated in monoculture (Fones and Gurr 2015; Palmer and Skinner 2002; Vurro et al. 2010; Zhu et al. 2000). The relationship between host density and disease prevalence has long been known, but density impacts in natural populations are often less drastic because patterns of host density and genetic diversity are more variable, hosts are found in mixture with other plant species, and disease severity can be modulated by environmental factors (Burdon and Chilvers 1982; Jarosz and Davelos 1995; Lively et al. 1995; Warren and Mordecai 2015).

Of the different groups of plant pathogens, fungal phytopathogens are the most numerous and pose the greatest threats to individual plant and population viability, and among these, foliar pathogens are particularly common and diverse (Jarosz and Davelos 1995). Prior to the past few decades, analyses of the impacts of fungal phytopathogens on natural populations were limited in the diversity of taxa studied (Alexander 2010). Despite the recent interest in infectious disease in natural plant populations, a large proportion of this work has been on exotic species, agricultural weeds (reviewed in Goss et al. 2020) and woody plants (particularly those with economic import or their relatives) (reviewed in Boyd et al. 2013 and Holdenrieder et al. 2004). Studies of native, non-economically important herbaceous plants have received far less attention (Alexander et al. 2007; Burdon et al. 2002; Carlsson-Granér 2002; Clay 1984; Ingram et al. 2018; Warren and Mordecai 2010).

The impacts of phytopathic fungi may be enhanced by climate change. For example, in an Asian alpine meadow, experimental warming increased pathogenesis on several naturally-occurring plant species (Liu et al. 2019). Similarly, a warming climate increased the abundance and proportion of pathogenic soil-borne fungi throughout the world (Delgado-Baquerizo et al. 2020). In the past 20 years, much of North America has experienced a warming trend with the magnitude of warming greater in more southerly regions. Wetlands and bogs in the southern Appalachian Mountains are projected to experience drying conditions with loss of rare endemic species and species disjunct from more northern regions (Schultheis et al. 2010). Impacts are expected to be particularly high in ecological island communities such as the high elevation balds of the southern Appalachians (Burdon et al. 2006; Cartright 2019, Cartright and Wolfe 2016). In the recent past, one of the characteristic and most charismatic species of these balds, *Lilium grayi* S. Wats. (Liliaceae), has experienced an epidemic of lily leaf spot disease caused by the *Lilium*-specific fungal phytopathogen, *Pseudocercosporella inconspicua* (G. Winter) U. Braun (Mycosphaerellaceae) (Barrett 2017; Bates 1998; Ingram 2013; Ingram et al. 2017; Ingram et al. 2018; Ingram and Levy 2020; Powell 2011). The disease causes plants to undergo premature senescence of aboveground plant parts and thereby limits seed production.

The potential climate impacts on high elevation southern Appalachian balds and the evidence of increasing impacts of a fungal phytopathogen on one regional endemic suggest that other species may experience similar disease-related problems. Here we report on a leaf spot disease of *Veratrum viride* Ait. (Melanthiaceae), a robust native perennial easily identified by its tall stature (1–2 m), large plicate leaves, and a large terminal inflorescence of greenish-cream, 6-merous flowers (Mulligan and Munro 1987). Populations are usually found in cool, moist to wet areas, in sun or shade. The species has a geographical range encompassing eastern North America and extending south in the Appalachians to northern Georgia (Kartesz 2022; Weakley 2020). Occurrences in Georgia, South Carolina, and Tennessee are scattered and limited to the higher mountains of the Blue Ridge physiographic province.

In 2013, a leaf spot disease on *Veratrum viride* was observed on Roan Mountain, Carter County, Tennessee (R. Ingram, Dow AgroSciences, pers. comm.). Conidia isolated from leaf spots appeared to be *Pseudocercosporella sublineolata* (Thüm.) U. Braun, a *Veratrum*-specific phytopathogen previously isolated from *Veratrum* species in Eurasia, Korea, and North America (Braun 1988, Shin 1998). Infection of leaves causes leaf spots characterized as circular to longitudinally elongated, initially appearing yellow-green then transitioning to brown-black in color, with a discolored surrounding zone (Petrak 1947). Examination of conidia can yield a definitive diagnosis. Conidia are 60–120 µm × 3.5–5(6) µm, hyaline, arched with tapered ends and 1–5 transverse walls (Kim and Shin 1999; Petrak 1947). On highly infected leaves, spots elongate and coalesce followed by leaf senescence. Similarly, highly infected plants undergo premature aboveground senescence, a process that may be mediated by the production of cercosporin, a photosensitizing systemic toxin produced by several related species (Daub and Ehrenshaft 2000; Goodwin et al. 2001; Gunasinghe et al. 2016; Świderska-Burek et al. 2020). Cercosporin breaks down host cell membranes to provide nourishment for the fungal hyphae.

The goals of this study were to determine if *Veratrum viride* has been impacted by leaf spot disease, whether *Pseudocercosporella sublineolata* is the likely causal pathogen, and to test for seasonal, geographic, and long-time temporal trends in disease prevalence. The approach was to conduct a detailed, one-year demographic assessment of leaf spot disease in two populations of *V. viride* on Roan Mountain, and to conduct a search for disease symptoms in herbarium specimens from throughout the U.S. range of the species.

MATERIALS AND METHODS

Study Site. The study was conducted on Roan Mountain, Carter County, Tennessee, the site where *Pseudocercosporella sublineolata* on *Veratrum viride* was initially reported. Plants from two populations of the open balds were surveyed; the populations are located approximately 435 m apart, between Round Bald and Engine Gap, at approximately 1700–1800 m elevation (Figure 1). Appropriate permits were acquired from the Cherokee and Pisgah National Forests and the Southern Appalachian Highlands Conservancy.

Population1 was located on the steep slope of Round Bald (36°06'28.3" N, 82°06'10.3" W) and comprised of approximately 200 *Veratrum viride* plants; Population2 was located on the north side of Engine Gap on a gradual slope near a tree line (36°06'21.8" N, 82°05'54.5" W) and encompassed approximately 300 *V. viride* plants. In both populations, individuals were sporadically distributed in clusters (Figure 1). Associated vegetation included: *Angelica triquinata* Michx., *Avenella flexuosa* (L.) Drejer, *Carex flexuosa* Muhl. ex Willd., *Cinna arundinacea* L., *Laportea canadensis* (L.) Weddell, *Lilium grayi* S. Wats., and *Rubus canadensis* L.

Demographic and disease analyses. Twenty plants from each population were chosen and tagged by selecting every fifth plant, along southwest–northeast transects. This approach resulted in a single tagged plant in some *Veratrum viride* clusters and multiple tagged plants in other clusters, depending on plant density. From June through September 2021, data were collected every two to three weeks from each population for a total of seven recording events. Data collection dates were; 19 June, 4–5 July, 24 July, 6 August, 22 August, 3 September, and 18 September, hereafter referred to as Time1 through Time7. To minimize the chance of researchers acting as transmission vectors, latex gloves were worn and disinfected with a dilute bleach solution before and after interacting with a plant, and shoes were disinfected before and after interacting with a population.

When plants were fully grown in July, plant height was measured and the number of leaves was recorded. Senescent leaves were not included in the leaf count tallies. Disease incidence data were collected as the presence or absence of the leaf spot disease symptoms typical of *Pseudocercosporella sublineolata*. Disease severity was assessed as the number of leaf spots per leaf counted on two leaves per plant, Leaf4 (numbered from the ground up) near the lower part of the stem, and Leaf10 on the upper part of the stem. Leaf spots were recorded as a direct count if discrete spots were present. As the season progressed and leaf spots expanded and merged, these leaf spots were recorded as coalesced spots, and if the disease progressed further and led to leaf wilt, a leaf was classified as senescent (Figure 2A, 2B). In addition, the overall condition of each plant was assessed using a five-point disease severity scale corresponding to the proportion of the entire plant experiencing symptoms, with no disease symptoms (score = 4) and decreasing in increments of 25% to a senescent plant (score = 0) (Figure 2C). A similar approach was used to quantify disease severity of related leaf spot diseases (Garcia-Guzman and Dirzo 2001; Ingram et al. 2018).

Disease signs. To confirm the visual diagnosis, *Veratrum viride* leaf spots suspected of a *Pseudocercosporella sublineolata* infection were sampled for spores (conidia) from some of the tagged plants. Samples were collected by pressing 2 cm^2 of translucent tape to the leaf epidermis, removing the tape, applying the tape to a cover slip that had a drop of acid fuchsin solution on it, and sealing the cover slip with clear nail polish (Ingram 2013; Ingram and Levy 2020). The acid fuchsin solution stains *P. sublineolata* spores magenta, increasing visibility under the microscope. In addition, the stain toxicity inhibits spore germination and thereby preserves the diagnostic structure. Samples were examined at 100× and 200× to visualize conidia and conidiophores. Species identification was based on the shape, size, and number of cells per conidia. Conidia morphology was similar in all samples and ten conidia from each of three plants were measured to quantify length and width using an ocular micrometer.

Data analysis. Plant height and leaf number were compared among and within populations and conidia sizes were compared among plants using one-way ANOVAs. We hypothesized that plants impacted by disease would experience premature leaf senescence. If correct, then the number of green leaves per plant was expected to decline as the season and disease symptoms progressed (healthy plants usually retain leaves until a frost). To test the hypothesis, the number of (green) leaves per plant was compared between populations using a repeated measures ANOVA with sample time as the repeated variable. The Huynh-Feldt-Lecoutre correction for deviations from sphericity was applied to adjust the F-value degrees of freedom. Significance was assessed using the Wilks' statistic to test for differences between populations, time effects, and a potential interaction. All analyses were carried out using SAS v. 9.4 (SAS Institute Inc., Cary, North Carolina).

The number of leaf spots per leaf was transformed into an ordered categorical variable with four states; no spots, discrete spots, coalesced spots, and a senescent leaf. The data for Leaf4 and Leaf10 were analyzed separately using general estimating equations as implemented by the GENMOD procedure in SAS using time as a repeated variable and population as a fixed variable. Significance was assessed with chi square tests. A similar analysis was used to test the hypothesis that disease severity increased over time.

To examine factors that can be used to predict disease status, stepwise logistic regression was used in which the response variable was the disease score at Time5. Time5 was chosen because a steep decline in the disease score occurred between Time4 and Time5, after which most plants had senesced. Predictor variables were population, plant height, number of leaves at Time2–Time4 (during which time disease symptoms went from minimal to severe), number of leaf spots on Leaf4 and Leaf10 at Time2–Time4, and disease severity score at Time3 and Time4.

The LOGISTIC procedure in SAS was used with model selection criteria of 0.15 for entry and 0.25 for elimination in the model (the final model was not sensitive to a range of entry and elimination criteria). The global model significance was assessed using the likelihood ratio chi square statistic and predictor significance was assessed using the Wald chi square statistic.

Retrospective analysis using herbarium specimens. An assessment of leaf spot disease symptoms in herbarium specimens of *Veratrum viride* was conducted to test three hypotheses, all of which were derived from prior observations of the prevalence of lily leaf spot disease in *Lilium canadensis* L. and *L. grayi* S. Wats. (Ingram et al. 2017). The hypotheses were that disease symptoms: (1) increase over the course of a growing season, (2) increased over time (from the late 1800s to the present) and, (3) are more common in the southern part of the range. To assess leaf spot disease symptoms on herbarium specimens, we searched online databases of the Consortium of Northeastern Herbaria, Mid-Atlantic Herbaria Consortium, and Southeast Regional Network of Expertise and Collections. Specimens were included in the data analysis only if there was an image that included at least one full size mature leaf, and state, county, and collection date were present. These criteria resulted in a database of 345 specimens from throughout the U.S. range of *V. viride*, i.e., from Maine to Georgia. There were no usable specimens from Ohio or South Carolina, two states from which there were few specimens. Plants were considered positive for leaf spot disease symptoms if the leaf spots had the diagnostic characteristics of infection by *Pseudocercosporella sublineolata* including a shape that was roundish with grayish margins.

To test the hypothesis that the leaf spot disease symptoms had increased over time, specimen collection dates were grouped into three eras; early (1850–1939), middle (1940–1979), and late (1980–2021), a grouping that resulted in comparable numbers of specimens per era (129, 125, and 91, respectively). An analysis of frequencies was conducted based on a 2 × 3 contingency table comprised of two disease symptom states (negative/positive) and three eras. The hypothesis that there was a geographical component to the prevalence of disease symptoms was tested in a similar manner, in this case grouping states into three geographical regions; New England (CT, MA, ME, NH, RI), Mid-Atlantic (DC, DE, MD, NJ, NY), and Southern Appalachian (GA, NC, TN, VA, WV). Similarly, the hypothesis that disease symptoms increased over the growing season was tested by grouping specimen collection dates into three seasons; spring (April–May), early summer (June–July), and late summer (August–September). Significance was assessed using Fisher's exact test. When the difference was significant, similar tests were conducted between pairs of groups to determine which differed. The exact boundaries of each of these groupings had no impact on assessing significance.

RESULTS

Demographic and disease analyses. When plants completed vegetative growth (at Time2), plants in the two populations were similar in height (Population1: Mean (s.d.) = 94.5 (34.8) cm; Population2: Mean = 101.0 (27.3) cm; ANOVA: $F = 0.43$; $df = 1, 38$; $p = 0.51$) and in numbers of leaves (Population1: Mean (s.d.) = 15.9 (4.5); Population2: Mean = 16.5 (4.9); ANOVA: $F = 0.16$; $df = 1, 38$; $p = 0.69$). Symptoms of leaf spot disease first appeared in July after which an increasing number of plants were symptomatic and by late July (Time3), only one plant in the entire sample was asymptomatic. Conidia were found in leaf spots from 12 plants (Figure 3). Conidia length and width were similar in the three plants for which they were measured (conidia length: ANOVA: $F = 1.85$; $df = 2, 27$; $p = 0.18$; conidia width: $F = 0.67$; $df = 2, 27$; $p = 0.52$) (Table 1) and the means and ranges were all similar to the range of 60–120 ×

3.5–5(6) µm in the species description of *Pseudocercosporella sublineolata* (Petrak 1947) (Table 1). In all cases where a length (or width) was outside the specific range, the corresponding length (width) was within the range

All disease symptoms experienced a progressive increase as the growing season progressed. The mean number of leaves per plant declined significantly over time but between Time4 and Time5, the decline was more extreme in Population1 which gave rise to a significant time × population interaction (Table 2A; Figure 4A). In Population1, the apparent increase from Time5 to Time6 was caused by increasing numbers of senescent plants at which time these plants were dropped from the count, and this loss is reflected in the lower sample size. If sample Time6 was excluded from the analysis, the result was similar in showing significance in the time effect and the time × population interaction. The number of leaf spots per leaf on Leaf4 and Leaf10 showed a pattern of decline over time similar to that of the number of leaves, with a increase over time but no evidence of differences between populations or a time × population interaction (Table 2B, 2C; Figure 5A, 5B). The disease severity score also declined significantly over time and the decline was similar between populations (Table 2D; Figure 4B). Nearly all plants had senesced by September 3, a date that was weeks before the average first frost (Figure 2C).

A model of the disease severity score at Time5 had predictive power (chi square = 13.8; df = 1; $p < 0.001$) with four significant predictor variables, all of which were leaf variables: (1, 2) the number of leaf spots on Leaf4 and Leaf10 at Time3 (Leaf4: chi square = 5.7; df = 1; $p = 0.02$; Leaf10 chi square = 3.1; df = 1; $p = 0.08$), (3) the number of leaf spots on Leaf4 at Time4 (chi square = 10.7; df = 1; $p = 0.001$) and, (4) the number of leaves at Time4 (chi square = 4.6; df = 1; $p = 0.03$).

Retrospective analysis of herbarium specimens. A total of 25 (7.2%) of the 345 herbarium specimens showed symptoms of leaf spot disease. There was no evidence that symptomatic plants were more common in the more recent eras (Table 3A) but there was an increasing prevalence of symptomatic plants as the growing season progressed (Table 3B) with no plants displaying symptoms in the spring. Disease prevalence in each of the seasons was significantly different from the others ($p < 0.01$ for each pair). There were also significant differences in disease prevalence among the geographical regions (Table 3C) with significantly higher prevalence in the mid-Atlantic and southern Appalachian regions compared to New England ($p = 0.02$ and $p < 0.01$, respectively) but the former two were not significantly different from each other ($p = 0.64$).

The two earliest symptomatic specimens were both from the mid-Atlantic region (U.S.A. MD: Garrett, 1 Aug 1904, *Norton s.n.* MARY) and (U.S.A. NJ: Ocean, 7 July 1907, *Grove 532* PH); the earliest symptomatic specimens from the southern Appalachians were collected 25+ years later, in the 1930s (U.S.A. TN: Carter, 10 July 1934, *Brown 93* DUKE; VA: Fauquier, 6 June 1935, *Allard 621* VPI); and the first (and one of only three) symptomatic specimen from New England was not collected until 1966 (U.S.A. NH: Coos, 6 Aug 1966, *Harris 29829* NEBC). The 1934 Tennessee specimen was collected from Roan Mountain, the site of our demographic and disease analysis.

DISCUSSION

Plants of *Veratrum viride* experienced a progressive decline during the growing season as evidenced by the loss of leaves and the increase in the number of leaf spots. Once the leaf spot disease was established in a population, the progression to leaf and whole plant senescence was rapid and ubiquitous. Consequently, there were no predictors of decline that were not based on prior disease status, that is, none were based on early season characters such as plant size, leafiness, or population. All plants in both populations were susceptible to the leaf spot disease but there were subtle differences in susceptibility. On three plants, the disease symptoms on one of the two sample leaves had not progressed to senescence, and on two other plants, both sample leaves had not progressed to senescence. All of these less symptomatic plants were on the northeast end of Population2. A similar pattern of disease clusters was found for the lily leaf spot disease on Roan Mountain where the regions with clusters of less susceptible plants were stable over three seasons (Ingram et al. 2017). The apparent clustering of less susceptible plants suggests either some level of innate resistance or a location in an environment less favorable to the pathogen, but high rates of autoinfection (within-plant inoculation of new sites) may also lead to disease severity clusters in foliar diseases (Mundt 2009).

Leaf and plant senescence was likely caused by production of cercosporin by *Pseudocercosporella sublineolata*. Once thought to be restricted to species in the genus *Cercospora* (Goodwin et al. 2001), the cercosporin toxin was recently isolated from *P. capsellae* (Ell. and Ev.) Deighton—a Brassicaceae-specific leaf spot disease—and other non-*Cercospora* members of the Mycosphaerellaceae (Gunasinghe et al. 2016; Świderska-Burek et al. 2020). Anecdotal reports suggest that *Veratrum viride* plants in the open sun of a bald are more severely diseased compared to plants in full or partial shade. Plausible explanations would include a higher level of stress (higher and more extreme fluctuations in temperature, exposure to UV radiation) or more intense sunlight-mediated effects of the photoactivated cercosporin toxin (reviewed in Daub and Ehrenshaft 2000). Production of cercosporin can additionally vary dependent upon the fungal strain, light, and (in the lab) culture medium (Jenns et al. 1989).

Moreover, some bacteria can degrade cercosporin (Taylor et al. 2006). Although the hypothesis that modulation of cercosporin production by light is an attractive one to also explain perceived differences between sun and shade populations, in *Lilium grayi* infected with *P. inconspicua*, clusters of plants showing low disease impacts were found in parts of the sunny bald habitat (Ingram et al. 2018).

Conidia and conidiophores viewed on slides from each of the 12 *Veratrum viride* plants examined were almost exclusively of one type, with the morphology corresponding to that of *Pseudocercosporella sublineolata*. The single spore type observed on *V. viride* was notably different from the diversity of spore types on slides prepared in a similar manner from *Lilium grayi* on Roan Mountain. Many of those slides contained spores of more than one type, often dominated by *P. inconspicua* but after spring there were also spores of species presumed to be opportunists and commensals (Ingram et al. 2018; Ingram and Levy 2020). Moreover, completion of Koch's postulates provided definitive evidence that the causal organism of the lily leaf spot disease was *P. inconspicua* (Ingram and Levy 2020). In this study, we have not completed Koch' postulates but the purity of the isolates, the conidia with diagnostic morphology, and the characteristic disease symptoms strongly implicate *P. sublineolata* as the causal organism.

Historic occurrences of presumed leaf spot disease on *Veratrum viride* were first observed on specimens from the mid-Atlantic and southern Appalachian regions, decades before the appearance of a specimen from New England; the former two regions have maintained higher frequencies into current times. The regional difference in the earliest symptomatic specimens was evident despite a higher number of pre-1940 specimens from New England. The pattern of earliest occurrence and high prevalence in the more southern parts of the geographical range suggest the disease is a more recent arrival to the northern region. In contrast, the prevalence of lily leaf spot disease in the southern Appalachians was high in the recent past but the earliest occurrences of the disease were from New York and New England, and therefore suggested dispersal from north to south (Ingram et al. 2017).

The prevalence of *Veratrum* leaf spot disease estimated from the herbarium survey is likely an underestimate because the majority of specimens in databases were collected in the early to mid season when plants are fresh appearing and flowering, rather than in late season when the disease symptoms become more apparent—two thirds (66.5%) of specimens in the sample were from April–June, a period during which only 20.0% of the disease cases were noted. Also, because plants are large, herbarium sheets often preserve only a few mature leaves.

Based on the natural geographic range and occupied sites, *Veratrum viride* is a species that prefers cool, moist climates (Mulligan and Munro 1987). The Roan Mountain survey showed that *V. viride* is clearly susceptible to *Pseudocercosporella sublineolata* infection. The apparent lesser susceptibility in New England may be a consequence of host resistance factors, greater host vigor in a more favorable environment, lesser pathogen virulence, an interaction of these factors, or failure of the pathogen to have dispersed sufficiently in that region. Hence, it is not possible at this time to assess the potential impact of climate change on this host-pathogen system.

Disease reservoirs can reside in alternate host species and these reservoirs can stymie attempts to mitigate disease impacts. The role of alternate hosts has been studied most intensively in wild and weedy species that may serve as disease reservoirs for crops (Wisler and Norris 2005). These interactions can be particularly important at the crop-natural system interface (Burdon and Thrall 2008). A wild plant/rare plant reservoir system was highlighted in

Lilium species. In *L. grayi*, a southern Appalachian endemic with dispersed populations, lily leaf spot disease has been found throughout the geographical range. Based on the number of populations impacted and disease prevalence, the disease was considered an epidemic posing a threat to species viability (Barrett 2017; Ingram et al. 2018). The potential threat of lily leaf spot disease is exacerbated by a disease reservoir in the highly susceptible *L. superbum* L., a species with a broader geographical range, wider ecological amplitude, and often found in high numbers in populations (Barrett 2018; Weakley 2020). Similarly, a disease reservoir for *Veratrum viride* may exist in the related *Melanthium parviflorum* (Michx.) S. Wats. (formerly *V. parviflorum* Michx. [Liliaceae]), also a southern Appalachian endemic but with a broader geographical range within that region compared to *V. viride* (Kartesz 2022). Specimens of *M. parviflorum* with leaf spots similar to those caused by *Pseudocercosporella sublineolata* on *V. viride* were common in online databases. However, the reservoir hypothesis must be considered tentative because in *M. parviflorum* we have not confirmed the disease diagnosis via a spore analysis.

In the two populations studied, infection of *Veratrum viride* by a leaf spot disease was ubiquitous and caused premature senescence of leaves and stems. Disease symptoms and signs showed the causal pathogen was *Pseudocercosporella sublineolata*. At best, there was scant evidence that some plants could escape devastating effects of the leaf spot disease. Unlike the lily leaf spot of *Lilium grayi* and *L. canadense*, the *V. viride* leaf spot does not appear to have become epidemic but it is more common in the southern part of the range. However, with a warming climate, the *Veratrum* leaf spot disease may become a more widespread problem over time.

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Table 1. Mean (standard error of the mean in parentheses) conidia length and width in three plants of *Veratrum viride* based on measurements of ten conidia per plant.

	Plant1	Plant2	Plant3
Conidia length (μm)	97.2 (7.3)	89.7 (6.2)	79.5 (6.1)
Conidia width (μm)	5.6 (0.5)	6.1 (0.5)	6.5 (0.6)

Table 2. Results of repeated measures ANOVA and general estimating equation analyses on the influence of time and population on disease indicators in two populations of *Veratrum viride* on Roan Mountain, Carter County, Tennessee. The number of leaves per plant does not include senescent leaves. An F-ratio was the test statistic for number of leaves per plant chi square for the other analyses.

	F	df	*p*
A. Number of leaves per plant			
time	15.9	4,12	< 0.0001
population	2.9	1,15	0.11
time × population	7.2	4,12	< 0.01
	χ^2		
B. Number of leaf spots: Leaf4			
time	33.4	3	<0.0001
population	1.3	1	0.27
time × population	6.7	3	0.08
C. Number of leaf spots: Leaf10			
time	34.4	3	< 0.0001
population	1.0	1	0.96
time × population	0.1	3	0.10

D. Disease severity			
time	40.0	4	< 0.0001
population	1.7	1	0.19
time × population	4.6	3	0.21

Table 3. Frequency of herbarium specimens of *Veratrum viride* with and without symptoms of leaf spot disease arranged by; (A) era, (B) season, and (C) region of occurrence. Percent symptomatic is shown in parentheses. The *p*-value associated with Fisher's exact test comparing frequencies is shown at the bottom of each section.

	Symptoms	
	Absent	Present
A. Era		
1850–1939	121	8 (6.2%)
1940–1979	117	8 (6.4%)
1980–2021	82	9 (9.9%)
total	320	25
Fisher's exact $p = 0.35$		
B. Season		
Spring	107	0 (0%)
Early Summer	201	19 (8.6%)
Late Summer	12	6 (33.3%)
total	320	25
Fisher's exact $p < 0.0001$		
C. Region		
New England	139	3 (2.1%)

Mid-Atlantic	69	7 (9.2%)
Southern Appalachian	112	15 (11.8%)
total	320	25
Fisher's exact $p < 0.01$		

Figure Legends

Figure 1. Map showing the location of the two study populations of *Veratrum viride* on Roan Mountain. Arrows represent tagged plants in each population. The star on the inset map shows the location of Roan Mountain, Carter County, Tennessee.

Figure 2. Photographs *Veratrum viride* with symptoms leaf spot disease showing, (A) a leaf with early-mid stage symptoms characterized by discrete leaf spots, (B) a leaf with mid-late stage symptoms characterized by coalescent leaf spots (arrows point to leaf spots visible in each of the discolored ribs), (C) a plant undergoing leaf and stem senescence (note vigorous neighboring plants showing no evidence of frost damage).

Figure 3. Photograph of conidia of *Pseudocercosporella sublineolata* isolated from a leaf spot of *Veratrum viride* on Roan Mountain, Tennessee. Conidia were stained with a dilute solution of acid fuchsin. Diagnostic characters include a length of 60–100 µm, fusiform shape, and 2–5 transverse walls per conidium. Circular structures may be remnants of conidiophores. Scale bar = 1000 µm

Figure 4. Plot of; (A) mean number of leaves from Time2 to Time6 and, (B) mean disease severity score from Time3 to Time7, both shown for each population of *Veratrum viride* on Roan Mountain, Tennessee. Error bars represent standard errors of the mean.

Figure 5. Plot of number plants in four categories of leaf spot disease symptoms on *Veratrum viride*. X-axis shows disease category distribution from Time3 to Time6 for, (A) Leaf4, and (B) Leaf10. Data from two populations combined.