Exobasidium leaf and fruit spot development in southeastern environments and development of initial management strategies with fungicides

Final report; *Research proposal 2012-01*

Principal Investigators:

Phillip M. Brannen
Extension Plant Pathologist – Fruits
Department of Plant Pathology
2106 Miller Plant Sciences
University of Georgia
Athens, GA 30602
Phone: (706) 542-1250
FAX: (706) 542-4102
Email: pbrannen@uga.edu

Bill Cline
Extension Plant Pathologist
Department of Plant Pathology
North Carolina State University
Horticultural Crops Research Station
3800 Castle Hayne Rd
Castle Hayne, NC 28429-6519
Phone: (910) 675-2314
FAX: (910) 675-0242
Email: bill_cline@ncsu.edu

Harald Scherm
Professor
Department of Plant Pathology
2105 Miller Plant Sciences
University of Georgia
Athens, GA 30602
Phone: (706) 542-1258
FAX: (706) 542-1262
Email: scherm@uga.edu

Marin Brewer
Assistant Professor
Department of Plant Pathology
2105 Miller Plant Sciences
University of Georgia
Athens, GA 30602
Phone: (706) 542-1254
FAX: (706) 542-1262
Email: mtbrewer@uga.edu

Objectives: (1) understand in-season disease progress and symptom development over time; (2) identify the efficacy of fungicidal classes against this disease, as well as approximations of critical application periods that would correspond with epidemic development; and (3) characterize the species of *Exobasidium* causing fruit and leaf spot on southeastern blueberries through an isolate survey.

Justification: Exobasidium fruit and leaf spot has been a sporadic and geographically dispersed disease of blueberries in the Southeast. However, during the 2011 season, the disease was frequently reported in rabbiteye and highbush blueberries. The fruit stage of this disease was first identified in 1997 in North Carolina (Cline 1998). The disease has since been observed throughout the Southeast, and it is closely related, in its causal agent and symptoms, to Exobasidium leaf and fruit spot described on lowbush blueberry in Canada (Nickerson & Vander Kloet 1997). The causal agent is also related to red leaf disease that occurs on lowbush and highbush blueberries as far north as Canada (Caruso & Ramsdell 1995); however, the disease cycle and pathogen species involved likely differ between the two diseases.

Though scattered, where Exobasidium leaf and fruit spot occurs it can cause significant losses (60-70% in specific locations), primarily because affected fruit are unmarketable and it is difficult to remove all berries with this symptom from the packing line (Fig. 1). Symptoms on rabbiteye blueberries in Mississippi have been so severe and recurrent as to cause fields to be abandoned. Exobasidium-affected berries can be eaten, but they are not palatable. Although the fruit spots do not rot or become necrotic, they do not ripen well and remain firm and green.

The cause of Exobasidium fruit and leaf spot is currently not identified to species level, apart from being placed within the genus *Exobasidium* (Nickerson & Vander Kloet 1997). Other related species, such as the pathogens causing red leaf disease of blueberry or leaf gall of azalea, are grouped under *Exobasidium vaccinii*, which describes a species complex (Caruso & Ramsdell 1995; Sinclair & Lyon 2005). It is currently unknown how the *Exobasidium* sp. causing leaf and fruit spot relates to *E. vaccinii* and other species within this complex.

Little is known about the disease cycle. Infection occurs early in the spring, and unlike some other Exobasidium diseases, infections are not systemic. Late-season leaf flushes do not show additional spots. With the *Exobasidium vaccinii* that causes leaf galls on azalea, the spores produced from leaves blow or wash to flower or leaf buds to infect anew, but symptoms of galling do not occur till the following spring. High humidity and excess moisture, especially as related to poor air flow and proximity to standing water or ponds, has been observed to influence the incidence and severity of the disease.

We know little about managing this disease, apart from a one-year field trial at Mississippi State University conducted by Dr. David Ingram in 2008. In his trial, Pristine performed best against both the leaf and fruit spot phases of the disease, followed by Elevate. No information was developed as to fungicide timing in relation to the disease cycle or epidemiology. The studies conducted here will start to address critical information for informed management of this disease.

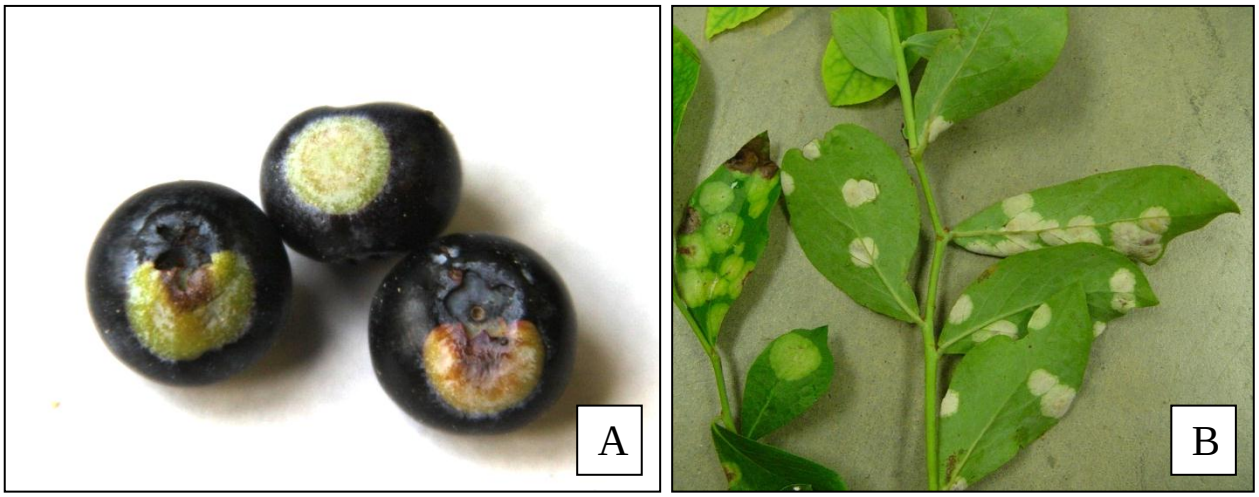

Figure 1. Symptoms of Exobasidium fruit (A) and leaf spot (B). Fruit symptoms are green, firm spots and blotches that do not mature with the rest of the berry. Leaf symptoms are light green spots on the upper leaf surface that are white or lighter green on the lower surface (photos courtesy of Eddie McGriff, University of Georgia, and David Ingram, Mississippi State University).

Methodology:

Field trials. Two field trials were conducted in Georgia (one each in Bacon and Coffee counties) on mature 'Premier' cultivar plants. Treatments were designed to address both application timing and efficacy of materials for Exobasidium fruit and leaf spot management (Table 1). Four fungicides from different mode-of-action classes (Captan, Elevate, Indar, and Pristine) were applied in full (F), early (E), mid (M), and late (L) schedules; the full schedule had a total of 9 applications, whereas each of the partial schedules consisted of 3 applications. The E schedule coincided with the bloom application window typically used for managing mummy berry disease (Table 1). The partial schedules had non-overlapping sprays. A randomized complete block design was utilized; five replications of each treatment were executed, and each replicate contained 10 plants. Fungicide applications were made with a standard air-blast sprayer using an application volume of 40 gal/acre. Applications were made to both sides of the bush. A minimum of two rows were skipped between spray rows to minimize plot-to-plot spray drift. Untreated control plots (no fungicides applied) were monitored in detail for the disease. Incidence of symptomatic leaves was assessed twice, while disease incidence on fruit was assessed once. The number of symptomatic leaves per plant was counted in each plot in mid to late April (before the L schedule commenced) and again in late May (after all fungicide applications had been made); leaves were counted on one side of the bush, extrapolating to the entire plant. In mid-May, after the completion of all fungicide sprays, a random sample of 250-350 fruit per plot was sampled and assessed for incidence of Exobasidium fruit spot (proportion of fruit with symptoms where 1.0 corresponds to 100%).

For the Coffee County site, square root transformation of the leaf count data and arcsine-square root-transformation of the fruit incidence data was utilized for statistical data analysis. For the Bacon County site, the appropriate data transformations were $\log_{10}$ for symptomatic leaf counts and arcsine-square root for fruit incidence. For a trial with so many treatments (total of 17, Table 1), a conventional means separation analysis was inappropriate, hence all treatments were compared with the untreated control (Dunnett's test) and orthogonal contrasts were utilized to compare the different timings at a significance level of $P = 0.05$ (see Figs. 2 and 3).

Pathogen isolate survey and characterization. Disease samples were collected in the aforementioned field trials from both leaves and fruit. Additional samples were obtained from natural outbreaks in GA and NC via county agent collaborators. Furthermore, we obtained additional *Exobasidium* isolates from other *Vaccinium* spp. from the eastern U.S. and Canada. Pathogens were isolated onto potato dextrose agar (PDA) by cutting fruit or leaf spots from tissue with a sterile scapel, affixing the cut tissue to the lid of a Petri dish with petroleum jelly and transferring to a fresh plate germinating basidiospores ejected onto the agar surface. Identification of *Exobasidium* was based on a detailed description of colony morphology and images published by Nagao et al. (2006). Isolates were purified and stored on in 30% glycerol at -80 ºC. To determine the relationship of the fungus causing Exobasidium fruit and leaf spot of blueberry in the southeastern U.S. with other *Exobasidium* spp., we sequenced the large subunit of the ribosomal DNA (LSU-rDNA) from nine isolates collected from fruit or leaf spots of rabbiteye (*Vaccinium virgatum* Aiton; formerly *V. ashei* Reade), northern highbush (*V. corymbosum)* and southern highbush (*Vaccinium* hybrid) blueberry from Georgia and North Carolina. Isolates of *Exobasidium* spp. causing leaf spot, red leaf disease, and gall on lowbush blueberry (*V. angustifolium*) were obtained from Maine and Nova Scotia and sequenced. We also obtained and sequenced isolates of *E. rotrupii* and *E. perenne* that cause leaf spot and red shoot disease of cranberry (*V. macrocarpon*), respectively. Sequences of *Exobasidium* spp. with high similarity to those causing fruit and leaf spots from the Southeast, as well as the type species of *E. vaccinii* from *Vaccinium vitis-idaea,* were obtained from the sequence database GenBank. The sequences

were assembled, edited, and aligned in Geneious Pro 5 and maximum likelihood was implemented in Mega 5 (Tamura et al. 2011) to construct the most likely phylogenetic tree.

Results:

Field trials. Both field sites had idiosyncrasies in the disease data. The Coffee County site (Fig. 2) had moderate-high disease pressure, but the untreated control plots consistently had very low disease. There were no significant differences from the control in leaf disease incidence, but during the first leaf assessment (16 April) meaningful comparisons could be made to the late (L) treatments which had not been sprayed at this time. The statistical contrasts were clear-cut: no difference between E and F, E significantly better than M or L, and no difference between M and L. Thus, at this site, early fungicide applications (treatments 6 through 9 in Table 1) were sufficient for disease management. There were significant differences from the control in fruit disease incidence, specifically Pristine E and F and Elevate E and F. The conclusions from the statistical contrasts were the same as for the leaf data. Most importantly, there was no significant difference between the E and F schedules, meaning that an early three-spray schedule performed as well as a full nine-spray schedule.

For the Bacon County site (Fig. 3), disease pressure was very high, and the untreated control consistently had high disease levels. Variability was lower, but the main idiosyncrasy here was that the best-performing chemicals from the other site (Pristine and Elevate) performed the poorest here. It is difficult to explain this result. The late (L) treatments did not add anything to disease control, whereas the mid (M) applications did somewhat, especially for the leaves. This may have been due to the fact that at this site disease initiation started a little later. For leaf disease control, Indar and Captan were by far the best chemicals and were significantly better than untreated. There is evidence at this site (based on the statistical contrasts) that the M sprays were better than the E sprays for leaf disease control. For fruit disease control, Indar E and F and Captan E and F were significantly better than untreated. Based on the contrasts, the M sprays did not add as much for the fruit disease.

Isolate survey. The sequence analysis phylogeny (Fig. 4) shows that isolates of *Exobasidium* sp. from blueberry in the Southeast are similar to, but distinct from, the *Exobasidium* sp. that causes leaf spots on lowbush blueberry, and are likely a different species. Both of these blueberry spots, however, are genetically different from other *Exobasidium* spp. that cause diseases on cranberry and blueberry, and from *E. vaccinii* from *V. vitis-idaea*. These results indicate that Exobasidium fruit and leaf spot of blueberry in the Southeast is caused by a unique species of *Exobasidium*. These results also suggest that within the Southeast this fungus is not genetically differentiated based on blueberry host species or cultivar, host tissue (fruit or leaf), or region (state), because the sequences do not cluster together. Results also show that sequences are more diverse than expected. To further investigate diversity within the Southeast, we sequenced the internal transcribed spacer region of the rDNA (ITS) from 73 isolates from diverse host species, cultivars, and locations in GA and NC. From the 73 isolates we obtained 64 unique sequences, which is an extremely high level of diversity and is unexpected for any fungus, let alone one causing an emerging disease. The high diversity indicates that the fungus causing Exobasidium fruit and leaf spot in the Southeast has existed for a very long time and that the recent increase in incidence is not a result of increased aggressiveness in the fungus or a recent host switch from another plant species, or a wild blueberry species to cultivated blueberries. Analyses of genetic differentiation of the ITS sequences confirmed our findings with LSU-rDNA that isolates within the Southeast are not differentiated by host species or cultivar, host tissue, or geographic region suggesting that a single population of this fungus is causing these symptoms on blueberry across the Southeast.

Table 1. Treatment regimens utilized to determine fungicide efficacy against Exobasidium leaf and fruit spot on 'Premier' rabbiteye blueberry in Bacon and Coffee counties, GA.

	Mummy Berry Sprays			Petal fall, Cover, and Pre-harvest Sprays					
	Green Tip (or early bloom)	Bloom Sprays (10-20% bloom)	Bloom Sprays (full bloom)	Petal Fall Spray (immediately after bloom)	Cover 1	Cover 2	Cover 3	Cover 4	Pre-harvest
1	NO FUNGICIDES APPLIED								
2	Pristine	Pristine	Pristine	Pristine	Pristine	Pristine	Pristine	Pristine	Pristine
3	Indar	Indar	Indar	Indar	Indar	Indar	Indar	Indar	Indar
4	Captan	Captan	Captan	Captan	Captan	Captan	Captan	Captan	Captan
5	Elevate	Elevate	Elevate	Elevate	Elevate	Elevate	Elevate	Elevate	Elevate
6	Pristine	Pristine	Pristine						
7	Indar	Indar	Indar						
8	Captan	Captan	Captan						
9	Elevate	Elevate	Elevate						
10				Pristine	Pristine	Pristine			
11				Indar	Indar	Indar			
12				Captan	Captan	Captan			
13				Elevate	Elevate	Elevate			
14							Pristine	Pristine	Pristine
15							Indar	Indar	Indar
16							Captan	Captan	Captan
17							Elevate	Elevate	Elevate

Treatments 6-9 were utilized to determine the percentage of disease control contributed by bloom sprays. Petal fall though second cover provided data to determine the contribution of mid-season sprays to disease management. Third cover through preharvest applications were utilized to determine the disease contribution from late-season infections. When compared to the full-season spray regiments, the comparisons to specific timing blocks (i.e. early, mid, and late) provided information on the contribution of each fungicide-timing block to disease control.

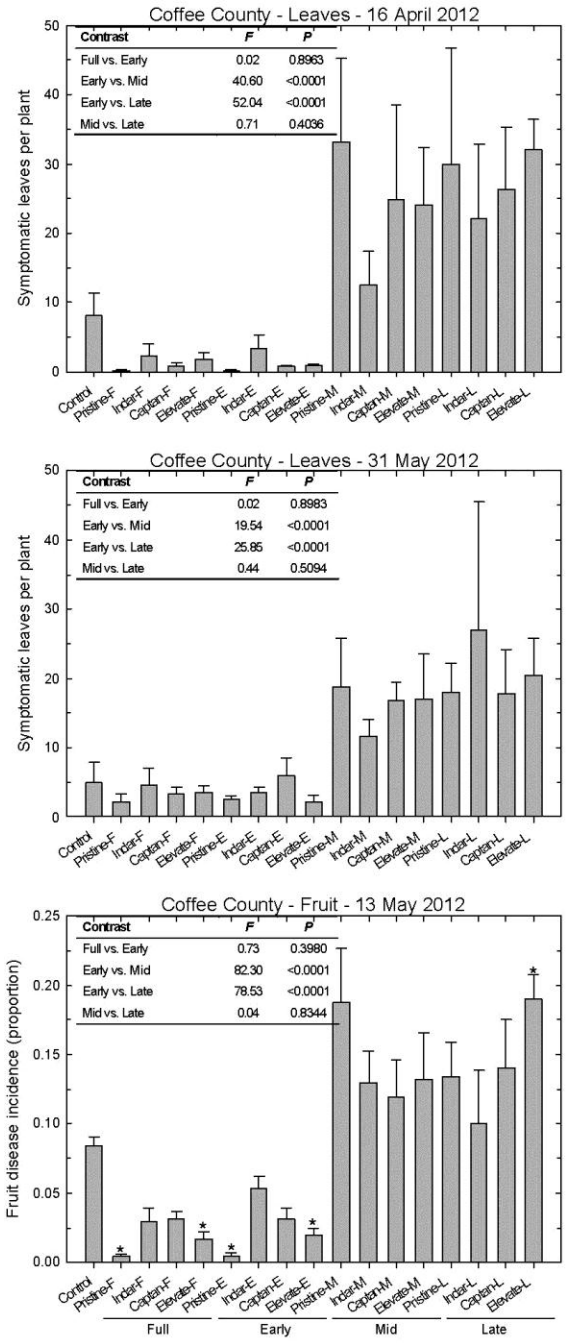

Figure 2. Exobasidium leaf and fruit disease incidence on 'Premier' rabbiteye blueberry in Coffee County, GA. Early (E), mid-season (M), and late (L) application schedules (three applications each) were compared with a full (F) schedule (nine applications) as shown in Table 1.

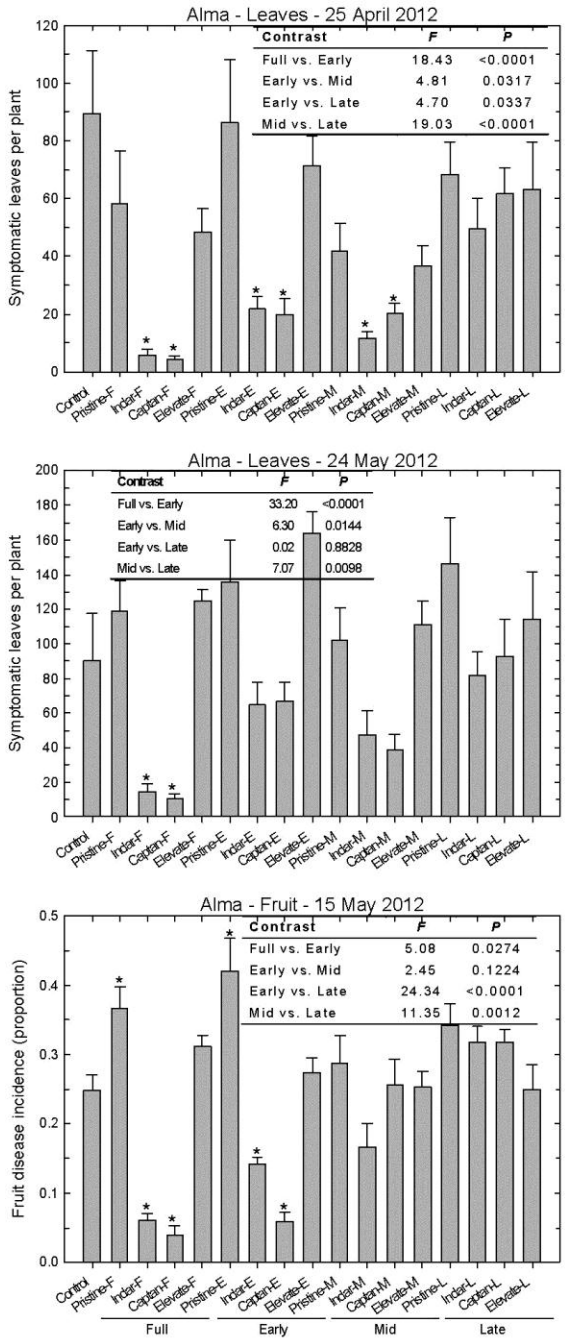

Figure 3. Exobasidium leaf and fruit disease incidence on 'Premier' rabbiteye blueberry in Bacon County, GA. Early (E), mid-season (M), and late (L) application schedules (three applications each) were compared with a full (F) schedule (nine applications) as shown in Table 1.

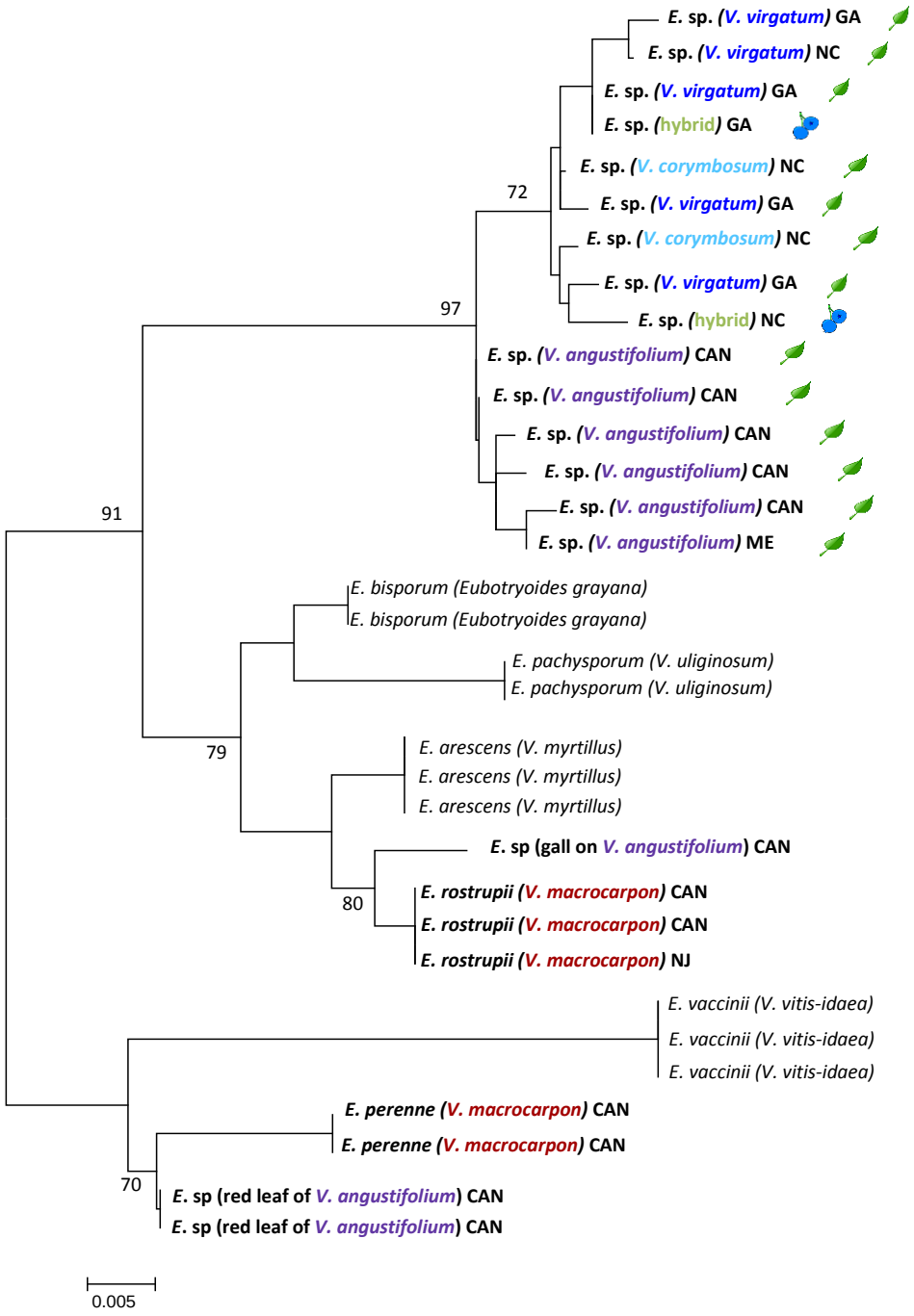

Figure 4. Most likely tree of the LSU-rDNA of *Exobasidium* sp. causing fruit and leaf spot of blueberry, high similarity sequences from related Exobasidium leaf spots and *Exobasidium* spp. causing other diseases of blueberry and cranberry. The host species is shown in parentheses. A leaf or blueberry symbol to the right indicates the host tissue from which the fungus was collected. Isolates that we sequenced are in bold and the region where the isolate was collected is indicated (GA = Georgia, NC = North Carolina, ME = Maine, CAN = Nova Scotia, Canada). Other sequences were obtained from GenBank. Branches with 70% or greater bootstrap support are shown.

Conclusions: The *Exobasidium* sp. associated with Exobasidium leaf and fruit spot is not new to the Southeast; any recent increase in observed disease is likely attributable to either inoculum buildup over time as the blueberry industry expanded, environmental responses, or changes in fungicides used (such as loss of benomyl, triforine, captafol, and folpet labels). The southeastern *Exobasidium* sp. is likely a different species from the *Exobasidium* sp. that causes leaf spots on lowbush blueberry and the red leaf disease in Canada and the northeastern U.S. The red leaf disease is caused by a systemic *Exobasidium* sp., and once a plant shows symptoms, the only recourse for the disease is plant destruction. The genetic and symptomatic distinctions between the leaf and fruit spot in the Southeast and the red leaf species indicate separate diseases. There is no current reason to recommend plant destruction when plants are infected with the southeastern species.

We can conclude from our field trials that early (green-tip through bloom sprays) are most important for managing the disease, but at least some component of the mid-season (petal fall to second cover sprays) may contribute to disease management. Additional research is needed to confirm these results, but if confirmed, the fungus is infecting in the earlier part of the season, possibly just following bud swell and during early leaf/bloom development. Anecdotal evidence has suggested that thorough fungicide programs for management of mummy berry disease will likely manage Exobasidium; Captan and Indar were consistently efficacious for management of Exobasidium in these trials, and both these fungicides are consistently applied during the green tip through petal fall timeframe. In addition, Pristine and Elevate are often applied during the same timeframe for management of mummy berry, Botrytis, and fruit rots; though the efficacy data for these fungicides was inconclusive, they may also contribute to disease management. The late-season sprays did not reduce disease at either site, so it is likely that later fungicide applications are of minimal importance for Exobasidium disease management, though they may be of value for management of fruit rot and leaf spot diseases. The infection of leaves appears to coincide with that of fruit, so the same fungicide schedule should likely control both leaf and fruit spots. Additional research is needed to further our knowledge of both the disease epidemiology and management.

Literature

1. Cline, W.O. 1998. An Exobasidium disease of fruit and leaves of highbush blueberry. Plant Disease 82(9):1064.
2. Caruso, F.L., and Ramsdell, D.C. eds. 1995. Compendium of Blueberry and Cranberry Diseases. American Phytopath. Society, St. Paul, MN.
3. Nagao, H., Exuka, A., Harada, Y., and T. Sato. 2006. Two new species of *Exobasidium* causing Exobasidium diseases on *Vaccinium* spp. in Japan. Mycoscience 47:277-283.
4. Nickerson, N.L., and Vander Kloet, S.P. 1997. Exobasidium leaf spot of lowbush blueberry. Canadian Journal of Plant Pathology 19:66-68.
5. Sinclair, W.A., and Lyon, H.H. 2005. Diseases of Trees and Shrubs, 2nd Ed. Cornell Univ. Press, Ithaca, NY.
6. Tamura, K., Peterson, D., Peterson, N. Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28:2731–2739.

Impact Statement: Results from this research indicate that the *Exobasidium* sp. that causes leaf and fruit spot of rabbiteye and southern highbush blueberries in the Southeast is likely a different species from its northern cousins infecting lowbush and northern highbush blueberries. It is not a new species to the Southeast or blueberries, so any recent increase in disease pressure is likely attributable to either inoculum buildup or environmental responses. Use of standard fungicides (Indar, Captan, and possibly others) for mummy berry and rot management during a period from green tip through early cover sprays will likely provide good management of Exobasidium, although additional research is needed for confirmation. Since this disease has caused substantive losses in some locations, we would anticipate that producers can immediately utilize the results of this research to reduce disease and increase production, while also reducing labor inputs associated with sorting out Exobasidium-infected fruit in the packing lines.