Title of Project: Clarifying the disease cycle of Exobasidium leaf and fruit spot of blueberry, with implications for practical disease management

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Objectives:
Previous collaborative research has identified the causal agent of Exobasidium leaf and fruit spot in the Southeast as *Exobasidium maculosum* (Brewer et al. 2014) and has resulted in empirically derived fungicide schedules to manage the disease (Scherm et al. 2014). However, the actual disease cycle is still largely unknown, resulting in uncertainty regarding our current control recommendations and lost opportunities for intervention with other possible disease management tactics. In this proposal, the overwintering biology of the pathogen will be clarified, and a value-added disease management tactics targeting the oversummering and overwintering stages will be evaluated. Specific objectives are to:

1) Quantify epiphytic overwinter survival of the yeast stage of *E. maculosum* on blueberry bark, leaves, and buds in naturally infected plantings.
2) Document that inoculum of *E. maculosum* applied to current season's growth during the typical period of basidiospore dissemination in spring will cause infection on leaves and fruit developing from buds on these twigs the following year in a planting with no prior history of the disease.
3) Determine the efficacy of fall applications of agrichemicals, applied on a typical schedule for fungal leaf spot control, to eradicate oversummering epiphytic inoculum of *E. maculosum* and thereby suppress Exobasidium leaf and fruit spot the following year.

Justification:
Exobasidium leaf and fruit spot (Fig. 1) has been reported from lowbush blueberry in Nova Scotia (Nickerson & Vander Kloet 1997) and from highbush blueberry in North Carolina (Cline 1998) during the 1990s. Until about 5 years ago, the disease has occurred only sporadically in the Southeast. Since then, however, the prevalence and intensity of the disease have increased considerably, with reports of significant losses (primarily on rabbiteye blueberry) from Georgia, North Carolina, and Mississippi (Brannen et al. 2011; Smith 2012). Heavily infected fields had to be abandoned in some cases. These economic losses are due primarily to the fruit infection stage, which renders the fruit unmarketable. Even with a low incidence of fruit infection, the sorting costs in the packinghouse can increase considerably.

Collaborative research during the past 3 years has revealed the following results about Exobasidium leaf and fruit spot:

- The disease is caused by the fungal pathogen *Exobasidium maculosum*, a new species described by Brewer et al. (2014). This pathogen has been isolated consistently from leaves and fruit of both rabbiteye and southern highbush blueberry throughout the Southeast.
- The disease appears to be monocyclic, i.e., there is a single infection cycle per year on both leaves and fruit. Furthermore, leaf and fruit infection appear to occur more or less simultaneously. This is deduced from the observation that fungicide schedules that control leaf infection also control fruit infection (Table 1), and that there is a good correlation between fruit and leaf infection across a wide range of fungicidal treatments.
- Spots on leaves (Fig. 1A) and fruit (Fig. 1C) produce sexual basidiospores of the fungus. These spores subsequently produce asexual blastospores (bud conidia) by budding. These yeast-like cells survive exceedingly well on dry and wet surfaces in the laboratory, suggesting that they may be the oversummering and overwintering structure of the pathogen in the field.
- Leaf spots become necrotic in July (Fig. 1B) and presumably no longer produce spores after this time.
- Our field trials (Scherm et al. 2014) showed that a single application of liquid lime sulfur at the delayed dormant stage controls both the leaf and fruit stages of the disease (Table 1). This is possible only if the pathogen overwinters in the bush, as opposed to being disseminated to blueberry plantings from external sources in the spring. Furthermore, this result suggests that the pathogen survives epiphytically on the plant surface (e.g., as long-lived blastospores).

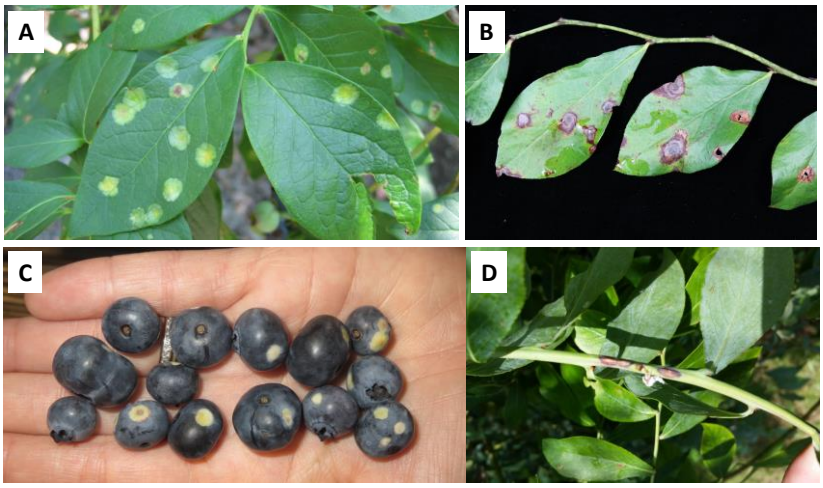

Fig. 1. Symptoms of Exobasidium leaf and fruit spot on leaves in early April-May (A) and later in July-August after desiccation of the lesions (B). Fruit symptoms (C) become visible in May-June (image courtesy Bill Cline, NCSU). Shoot lesions (D) form at the same time as leaf lesions, but their role in the disease cycle is unknown.

Based on the above observations, I propose the following disease cycle for Exobasidium leaf and fruit spot of blueberry: The pathogen survives epiphytically (on the plant surface or in shallow lesions) during summer, fall, and winter. The survival structure is the yeast-like blastospore. The pathogen may or may not multiply actively during this time. During winter and early spring, the blastospores are washed onto leaf and flower buds, where they get trapped on the bud scales. As the leaf buds break, the emerging leaves are infected directly. Blastospores from flower buds are transmitted to the flower and from there to the developing young fruit, where fruit infection occurs. Most likely two compatible blastospores will need to fuse before they can cause infection. Both leaf and fruit spots produce basidiospores of the pathogen, which are air-dispersed within and outside the blueberry planting. Once the basidiospores land on blueberry tissue (bark or leaves), they bud to form long-lived blastospores, which oversummer and overwinter to cause leaf and fruit infection the next spring, closing the disease cycle.

The experiments described herein were designed to test these hypotheses. Confirmation of the disease cycle would allow producers to focus their management efforts on the epiphytic blastospores, the presumed survival structure of the pathogen, particularly through preemptive, eradicant applications of effective fungicides.

Table 1. Influence of fungicides on incidence and severity of Exobasidium fruit and leaf spot on Premier rabbiteye blueberry in Bacon County, 2013 (Scherm et al. 2014).

Treatment and rate/A		Application timing[z]	Leaf spot incidence (%)[y]	Leaf spot severity (spots/leaf)[y]	Fruit spot incidence (%)[x]
1	Untreated control.....	----	48.0 a	2.9 a	32.4 a
2	Lime Sulfur 5 gal.....	1	5.7 cd	0.1 c	2.8 de
3	Captan 4L 2.5 qt.....	2-6	8.3 cd	0.2 c	4.4 cde
4	Indar 2F 6fl oz.....	2-6	23.1 b	0.5 c	12.3 bc
5	Pristine 22 oz.....	2-6	24.0 b	0.5 c	10.3 bcd
6	Captan 4L 2.5 qt.....	7-9	6.7 cd	0.1 c	12.2 bc
7	Indar 2F 6fl oz.....	7-9	15.5 bc	0.3 c	16.6 b
8	Pristine 22 oz.....	7-9	39.2 a	1.8 b	31.0 a
9	Captan 4L 2.5 qt.....	2-9	1.3 d	0.0 c	1.0 e
10	Indar 2F 6fl oz.....	2-9	4.4 cd	0.1 c	4.0 cde
11	Pristine 22 oz.....	2-9	25.0 b	0.7 c	14.4 b

[z]Treatment dates and corresponding plant phenology: 1 = 5 Feb (late dormant); 2 = 14 Feb (early green tip); 3 = 28 Feb (green tip, 4% bloom); 4 = 14 Mar (32% bloom); 5 = 21 Mar (58% bloom); 6 = 28 Mar (84% bloom); 7 = 1 Apr (petal fall); 8 = 12 Apr (first cover); 9 = 19 Apr (second cover).

[y]Recorded for 20 shoots per plot with ~7 leaves per shoot on average. Means followed by the same letter are not significantly different when using Tukey's test ($P = 0.05$).

[x]Recorded for ~300 fruit per plot on average. Means followed by the same letter are not significantly different when using Tukey's test ($P = 0.05$).

Methodologies:

Objective 1: The study was conducted on Premier rabbiteye blueberry in a commercial planting in Bacon County, GA. The site is comprised of mature (>10-year-old) plants and has a history of Exobasidium leaf and fruit spot. Our previous work has shown that *E. maculosum* populations at this site are insensitive to the active ingredients in the fungicide Pristine (pyraclostrobin + boscalid), allowing us to use Pristine-amended culture medium (0.5 ppm of active ingredients) for semi-selective pathogen isolation and tracking (Fig. 2). This is particularly important given the slow-growing nature of the pathogen in vitro. Our isolation medium also contains a relatively high concentration of dextrose (20 g/liter) as an additional selective agent since preliminary experiments have shown that *E. maculosum* is highly osmotolerant, as would be expected from an effective plant surface epiphyte.

At weekly to fortnightly intervals from 4 February through 13 April 2014 (pathogen overwintering phase), green bark, lignified bark, leaf bud, flower bud, and bark lesion samples were collected in the field and assayed in the laboratory for the presence *of E. maculosum* by

dilution-plating. Bark lesions are small, dark, raised areas formed on stems just below leaf or flower buds. At each sampling date, 25 samples per tissue type were excised with a flame-sterilized scalpel, placed individually in sterile test tubes, and transported to the lab on ice. Sterile water was added to each tube, and the suspensions were sonicated for 2 min and vortexed for 30 s. The wash water was dilution-plated in triplicate on our semi-selective Exobasidium isolation and enumeration medium. Petri dishes were incubated at 20^{o}C, and colonies of *E. maculosum* identified and counted as they emerged over a 3-week period based on characteristic colony morphology. The resulting data consisted of the mean population densities of the pathogen (as colony-forming units per g of tissue) on the surface of each tissue type from the dormant season to first symptom development.

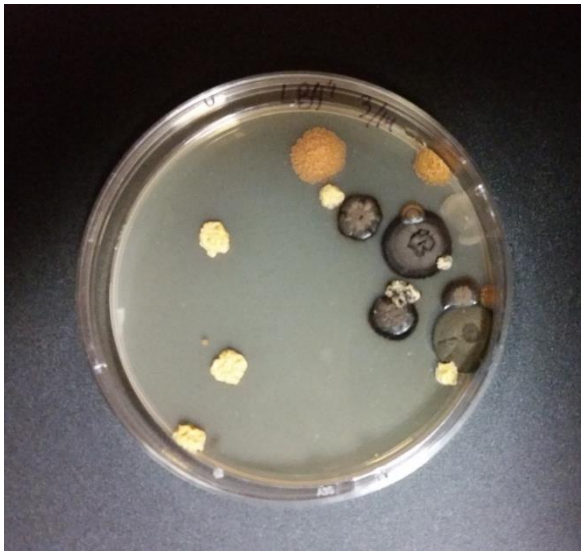

Fig. 2. Colonies of *Exobasidium maculosum* (cream-colored pure colonies and orange mixed colonies) from dilution-plating of blueberry leaf buds on semi-selective medium containing 0.5 ppm (a.i.) Pristine fungicide and 20 g/liter dextrose (pH 3.5).

Objective 2: Inoculation studies were undertaken at the UGA Horticulture Farm near Athens in an effort to induce disease development in a mature planting of cultivar Premier not previously observed to be affected by Exobasidium leaf and fruit spot. The experiment was designed to introduce inoculum into the field at a time corresponding to that at which natural inoculum would be present.

In 2013, six inoculations were made at 2-week intervals from mid-January until early April. Five replicate pairs of plants (inoculated and non-inoculated) were used at each date. Prior to each inoculation, ten 1-year-old shoots were selected and tagged, and the phenology of each vegetative and flower bud per shoot was recorded. During the 10-week period in which inoculations were made, vegetative buds advanced from stage 1 (dormant) to stage 6 (fully opened, expanding) and flower buds advanced from stage 2 (slight swelling) to late stage 5/early stage 6 (early bloom). In general, two isolates of *E. maculosum* (blastospores) obtained from leaf or fruit lesions in 2012 were cultured for 2 weeks in 100 ml of potato dextrose broth on a platform shaker to serve as inoculum. On the day prior to inoculation, cultures were removed from the shaker and allowed to settle overnight. Approximately half the liquid was decanted the next day and the remaining liquid agitated to resuspend the cells; the two cultures were then mixed together. The inoculum was applied to the tagged shoots with a 1-inch standard paint brush. Shoots on control plants received an application of sterile broth.

In 2014, individual bushes tagged in 2013 were reused. As previously, five two-plant replicates per inoculation date were used, although treatments were blocked within the orchard rather than distributed randomly. Shoots were selected and tagged as in the previous year, although phenological stage was assessed and recorded as a shoot average for both vegetative and flower buds rather than individually. At the onset of the experiment, which commenced in late February, most vegetative buds were at stage 2 and the majority of flower buds at stage 1. Shoots

were inoculated at 2-week intervals until early April, for a total of four treatment dates. The same isolates of *E. maculosum* were used as in 2013; however, isolates were cultured on potato dextrose agar dishes for 10-14 days and then washed with sterile water, combined, and suspended in a final volume of 500 mL in preparation for inoculation. The suspension was placed in an atomizing spray bottle and individual shoots treated; a handheld shield was employed to prevent inoculum deposition on untagged shoots. Adjacent bushes with tagged shoots served as untreated controls.

Additionally in 2014, blueberry shoots with actively sporulating lesions of *E. maculosum* were collected in a commercial field in southern Georgia in late May to early June. These shoots were attached onto expanding vegetative growth in the lower, shaded areas of several of the disease-free plants at the Horticulture Farm. Basidiospores from these lesions provided additional inoculum at a time coinciding between natural spore release and presumably susceptible plant tissues.

Table 2. Experimental treatments to determine the added benefits of fall or dormant-season applications of agrichemicals in suppressing Exobasidium leaf and fruit spot by reducing epiphytic inoculum, Bacon County. fall/winter 2014-15.

Trtmt. no.	Material and rate	Timing	Comments or added benefit of treatment
1	Untreated check	----	----
2	JPC Stylet Oil, 6 qt/100 gal	Late summer	Bud mite control
3	Captan 4L, 2.5 qt/acre	Two fall applications	Fungal leaf spot control
4	Lime sulfur, 5 gal/acre	Two fall applications	Fungal leaf spot control
5	Dormex, 0.75 gal/acre	Late dormant	Increase leafing and flowering
6	Superior oil, 3 gal/100 gal	Late dormant	Scale insect control
7	Lime sulfur, 5 gal/acre	Late dormant	Standard treatment

Objective 3: Two separate field trials were initiated in commercial rabbiteye blueberry plantings in Bacon County during fall 2014 to determine the added value of fall or dormant-season applications of agrichemicals in reducing oversummering or overwintering inoculum, respectively, of *E. maculosum* (Table 2). One trial utilizes cultivar Premier, whereas the other is being conducted on cultivar Alapaha. Treatments are being applied to eight-plant plots in a randomized complete block design using an airblast sprayer. Fall fungicide applications consisted of two sprays (early and late October) of Captan or lime sulfur, both of which are known to be effective against Exobasidium leaf and fruit spot when applied during the epidemic in the spring (Scherm et al. 2014). The rationale behind these two treatments was to determine whether typical fungicide sprays for fungal leaf spot control (such as Septoria or anthracnose) can add value against Exobasidium by suppressing oversummering cells. Another (single) fall treatment consisted of an early October application of stylet oil, mimicking a schedule for bud mite control. The rationale here was to determine whether oil coverage of the plant during the oversummering phase can suppress *E. maculosum* cells on the plant surface.

Subsequent dormant-season applications (mid-February 2015) will consist of a single application of lime sulfur, which has been shown to control the disease effectively in previous trials (Table 1); this will serve as a standard treatment for comparison. Other dormant applications will utilize Dormex (hydrogen cyanamide), which is used commercially to induce bud break and may have activity against overwintering surface inoculum of *E. maculosum* due to its caustic activity; and of dormant oil, which is used commercially against scale insects but may suppress overwintering cells of the pathogen through oil coverage of the plant surface (Table 2).

The expected outcome of this objective is the identification of agrichemical treatments with dual benefits, i.e., products such as oils or hydrogen cyanamide that are applied for a specific horticultural or pest management task but might have significant non-target activity against *E. maculosum* epiphytic inoculum. Knowledge of the added value of such applications could help improve management of Exobasidium leaf and fruit spot and/or reduce the number of fungicide applications needed subsequently, thereby lowering production costs.

Results

Objective 1: Surface inoculum of *E. maculosum* could be detected on most tissue types throughout the overwintering sampling period from early February through mid-April (Fig. 3). Isolation frequencies and population densities were consistently low on lignified bark and on flower buds. In contrast, numbers were highest on bark lesions and leaf buds. Peak numbers were observed from late February to mid-March on the latter two tissue types, i.e., during the bud break and bloom period.

Additional field observations in this test during spring and summer 2014 indicated that *E. maculosum* forms lesions on emerging young shoots in the spring (Fig. 1D), but there is no information on the fate of these lesions over time, specifically whether they develop into cankers and can serve as an oversummering and overwintering site. To shed light on this question, we tagged fresh, sporulating stem lesions with flagging tape as they emerged in the spring. These lesions will be observed during winter 2014-15 with a hand lens to determine presence or absence of sporulation. In January and February (during overwintering), and March (after overwintering), short stem sections with lesions will be excised with a sterile scalpel, placed in test tubes, and transported to the lab on ice. Small longitudinal sections will be taken from the lesions and examined microscopically for the presence of basidia and basidiospores, especially during the February and March sampling dates where they could serve as initial inoculum.

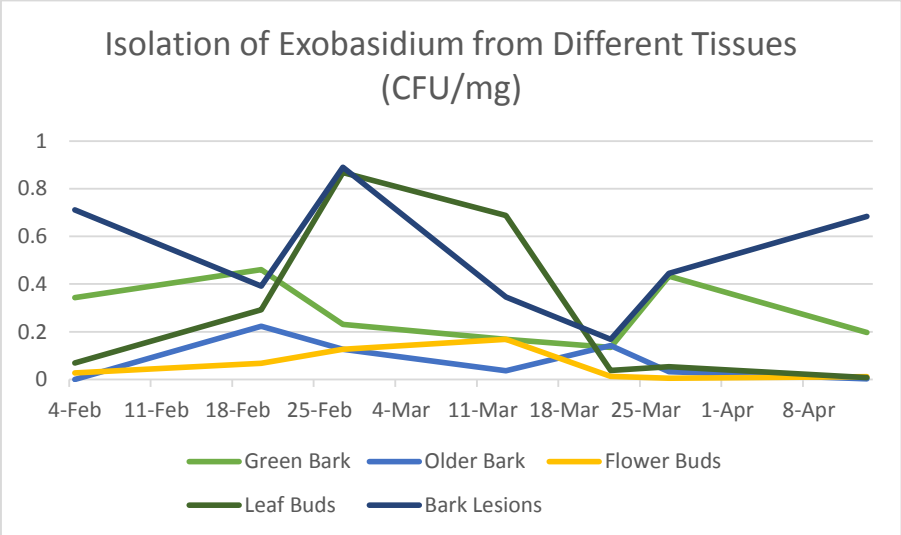

Fig. 3. Relative number of colony-forming units (CFU) of *Exobasidium maculosum* surface inoculum on Premier rabbiteye blueberry during the pathogen overwintering phase in 2014.

Subsequently all lesions will be surface-disinfested and tissue pieces from the margin between necrotic and healthy tissue will be plated on our semi-selective Exobasidium medium for pathogen confirmation.

Objective 2: No disease symptoms developed on inoculated plants in 2013, the year the planting was first inoculated. In 2014, one Exobasidium lesion on a single leaf was found, suggesting survival of epiphytic Exobasidium inoculum from 2013 to 2014. On plants inoculated in 2014 (either by spraying with lab-cultured blastospores or attaching of leaves with lesions producing basidiospores), no disease development was observed. These plants will be monitored again in spring and summer of 2015 to determine whether inoculum surviving from 2014 might cause infection.

Objective 3: The two trials are still underway. Fall treatments were applied in October 2014, and dormant applications will be made in mid-February 2015. Later in the spring of 2015, disease will be assessed in the center six bushes in each plot. Foliar disease incidence (proportion of affected leaves) and severity (average number of spots per leaf) will be determined in late April or early May based on 20 randomly selected shoots per plot. Fruit disease incidence will be determined before the first commercial harvest by randomly collecting >200 fruit per plot.

Conclusions

This epidemiological study has revealed several new features of the Exobasidium-blueberry pathosystem:

- The pathogen can be detected readily on plant surfaces during the winter and early spring in infected plantings, suggesting surface yeast inoculum (blastospores) plays a role in pathogen survival.
- Overwintering pathogen densities were highest on leaf buds and in bark lesions. This was the first time the inconspicuous bark lesions were implicated in facilitating survival of *E. maculosum*.
- Preliminary evidence for epiphytic survival was also obtained in our artificial inoculations where symptoms, albeit at a very low level, were observed in 2014 following inoculation in 2013 in a previously disease-free planting.
- Shoot infections by the pathogen were observed at the same time as leaf infections in the field. This is the first time that this symptom type was described formally for *E. maculosum*, but there is currently no information on the fate of these shoot lesions over time, specifically whether they develop into cankers and can serve as an oversummering and overwintering site.
- Field trials with fall and dormant applications of agrichemicals are currently underway to identify treatments with dual benefits, i.e., products such as oils or hydrogen cyanamide that are applied for a specific horticultural or pest management task but might have significant non-target activity against *E. maculosum* epiphytic inoculum.

Impact Statement

Situation

Exobasidium leaf and fruit spot has occurred only sporadically in the Southeast until about 5 years ago. Since then, the prevalence and intensity of the disease have increased considerably, with reports of significant losses (primarily on rabbiteye blueberry) from Georgia, North Carolina, and Mississippi. Heavily infected fields had to be abandoned in some cases. These

economic losses are due primarily to the fruit infection stage, which renders the fruit unmarketable. Even with a low incidence of fruit infection, the sorting costs in the packinghouse can increase considerably.

Response

CAES scientists, graduate students, and extension specialists are collaborating in field studies to better understand the disease cycle of Exobasidium leaf and fruit spot, especially the epidemiologically important oversummering and overwintering phases. In addition, agrichemical trials are being conducted to identify "dual-benefit" treatments, i.e., those applied for a specific horticultural or pest management task that might have significant non-target activity against oversummering or overwintering Exobasidium inoculum.

Impact

Our hypothesis that the pathogen survives on multiple plant surfaces during the winter was confirmed in this study, thereby providing a biological underpinning for the efficacy of lime sulfur applications during the dormant season. However, the study also revealed two new potential overwintering sites of the pathogen, bark lesions and shoot lesions. The role of these symptoms in pathogen survival needs to be clarified to ensure our current management recommendations are targeted optimally.

Citation(s) for any publications arising from the project

None this year.

References

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