Development of Latent Infections on Cranberry Leaves Inoculated with *Botryosphaeria vaccinii*

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ABSTRACT

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The range of dew period temperatures conducive to the establishment of latent leaf infections and the histopathological relationship of *Botryosphaeria vaccinii* to cranberry (*Vaccinium macrocarpon*) leaves were investigated in growth chamber tests with inoculated cuttings. Inoculated plants were placed in dew chambers at 16, 20, 24, 28, or 32 C for 72 hr then incubated at 20 or 28 C for 6 wk. Latent infections were established most often with a 28 C dew period followed by a 28 C incubation. Microscopic examination of inoculated leaves demonstrated conidial germination and appressorial development within 24 hr. Appressoria usually formed adjacent to germinating spores without prior germ tube elongation. Penetration pegs developed within 48–72 hr after inoculation followed by the formation of dormant infection hyphae within the cuticle or between the cuticle and outer wall of the epidermis. No additional development was noted up to 2 wk after inoculation.

Additional key words: *Guignardia vaccinii*.

Much attention has been given to latent infections of fruit of temperate and tropical crops as the cause of delayed pathogenesis (1,7,9,14,15). Less attention, however, has been given to latent infections of leaves that may also act as reservoirs of inoculum. Such knowledge is important in elucidating control strategies.

Botryosphaeria vaccinii (Shear) Barr (≡*Guignardia vaccinii* Shear; anamorph, *Phyllosticta elongata* Weidemann) is thought to establish latent or quiescent infections on cranberry (*Vaccinium macrocarpon* Ait.) fruit and leaves (2,5,6,12,17). Dormant fruit infections may result in a localized, superficial blemish (3,17), but further development is typically delayed until after harvest (13,17,18). Latent infections of leaves cannot be detected macroscopically and usually remain dormant until abscission occurs (5,17). Prior studies on latency in cranberry leaves have been based on isolations from naturally infected leaves (5,12,17), but latent leaf infections were not demonstrated conclusively by controlled inoculation, nor were the conditions necessary for establishment of latency known.

This investigation was conducted to determine the range of dew period temperatures conducive to establishment of latent leaf infections and to examine latent leaf infections histologically. Portions of this study have been published (16).

MATERIALS AND METHODS

Monoconidial isolates used in this study were obtained from naturally infected plant material from the Cranberry Experiment Station, East Wareham, MA 02538 (isolate F5), and the Blueberry and Cranberry Research Center, Rutgers University, Chatsworth, NJ 08019 (isolates F6 and F9). All isolates were maintained on potato-dextrose agar (PDA) slants at 4 C.

Cranberry plants (cultivar Searles) were propagated from dormant cuttings of uprights collected from a commercial marsh at Wisconsin Rapids, WI. Four plants were established in each 9-cm-diameter plastic pot containing peat, quartz sand, and unsterilized cranberry marsh soil (1:1:1, v/v). Plants were maintained in growth chambers (24 C, 16-hr photoperiod) and were pruned to 15–20 cm height 4–6 wk prior to use.

Conidial inoculum was prepared from cultures grown for 12–14 days at room temperature (22–24 C) on cellophane membranes on

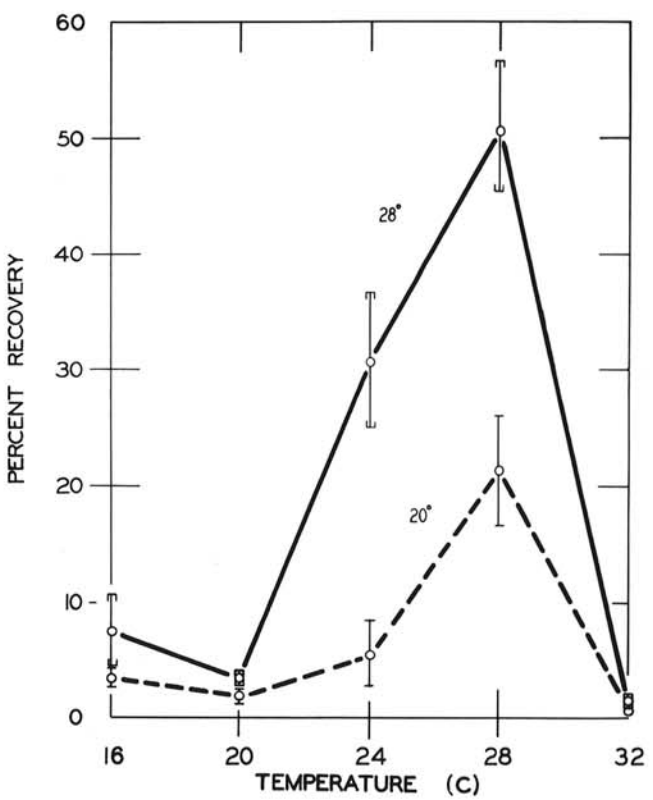

Fig. 1. Percent recovery of *Botryosphaeria vaccinii* (isolate F5) from symptomless inoculated cranberry leaves after 72 hr of incubation at five constant dew period temperatures (abcissa) followed by 6 wk of postinfection growth at 20 C (dashed line) or 28 C (solid line). Vertical bars are standard errors of the means.

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PDA plates as previously described (17). Conidial suspensions were standardized, with a haemocytometer to 10^5 conidia per milliliter for incubation temperature studies and 5×10^5 conidia per milliliter for histological studies. Plants were inoculated to runoff with an atomizer and placed immediately into darkened dew chambers at 16, 20, 24, 28, or 32 C. After 72 hr, half of the pots were transferred to a 20 C growth chamber (12-hr photoperiod) and the other half to a 28 C growth chamber (12-hr photoperiod). Six weeks after inoculation, 40 leaves of varying ages were randomly selected from the cuttings in each pot, excluding growth subsequent to inoculation. Leaves were surface-disinfested for 5 min in 1% sodium hypochlorite (NaOCl) plus one drop of Triton X-100 per 100 ml of solution, rinsed in sterile distilled water, and plated on PDA. The plates were incubated for 10–14 days at room temperature (22–24 C). Each treatment was replicated three times with a single pot of four plants per replicate.

Appressorial development on leaf surfaces was determined 24, 48, and 72 hr after inoculation, with polystyrene leaf replicas (6% polystyrene in 2:1 benzene:toluene) mounted in lactophenol-aniline blue. Adaxial and abaxial leaf surfaces were examined.

For histological studies, plants were inoculated as described above, placed immediately in a darkened dew chamber at 24 C, and after 72 hr were transferred to a 24 C growth chamber (16-hr photoperiod). Samples of 10–15 leaves were collected 1, 2, 3, 7, and 14 days after inoculation. Leaves were cleared in boiling 95% ethanol for 10 min, heated in 5% sodium hydroxide for 10 min, rinsed with distilled water, and soaked in 15 M chloral hydrate for 2 hr. The leaves were examined under a light microscope, and areas with an abundance of appressoria were excised, fixed in FAA, dehydrated in a *t*-butanol series, and embedded in paraffin (11). Sections (12 μm) were cut with a rotary microtome and mounted in lactophenol-aniline blue. Microscopic examinations were made with bright-field and Nomarski optics.

RESULTS

B. vaccinii was recovered from symptomless, surface disinfested cranberry leaves 6 wk after inoculation at all dew period temperatures that were tested (Fig. 1). Best establishment occurred after a 28 C dew period followed by a 28 C postinfection incubation. Postinfection establishment was consistently greater at 28 C than 20 C.

Conidial germination and appressorial formation was observed on both adaxial and abaxial leaf surfaces 24 hr after inoculation (Fig. 2A,B). The proportion of germinated spores producing appressoria increased from 27% at 24 hr to a maximum of 70% at 72 hr. Appressoria usually formed adjacent to germinating conidia without prior germ tube elongation (Fig. 2A,C), although occasionally spores developed a short germ tube prior to appressorial development (Fig. 2B). Formation of appressoria was not consistently associated with stomata on abaxial surfaces or with the anticlinal walls of epidermal cells.

Penetration pegs that developed from appressoria were first observed 48 hr after inoculation (Fig. 2D). By 7 days, a swollen infection hypha formed within the cuticle or between the cuticle and outer wall of the epidermis (Fig. 2E,F). The cell wall matrix surrounding the infection hyphae was more refractile than uninfected areas when observed with bright-field optics and

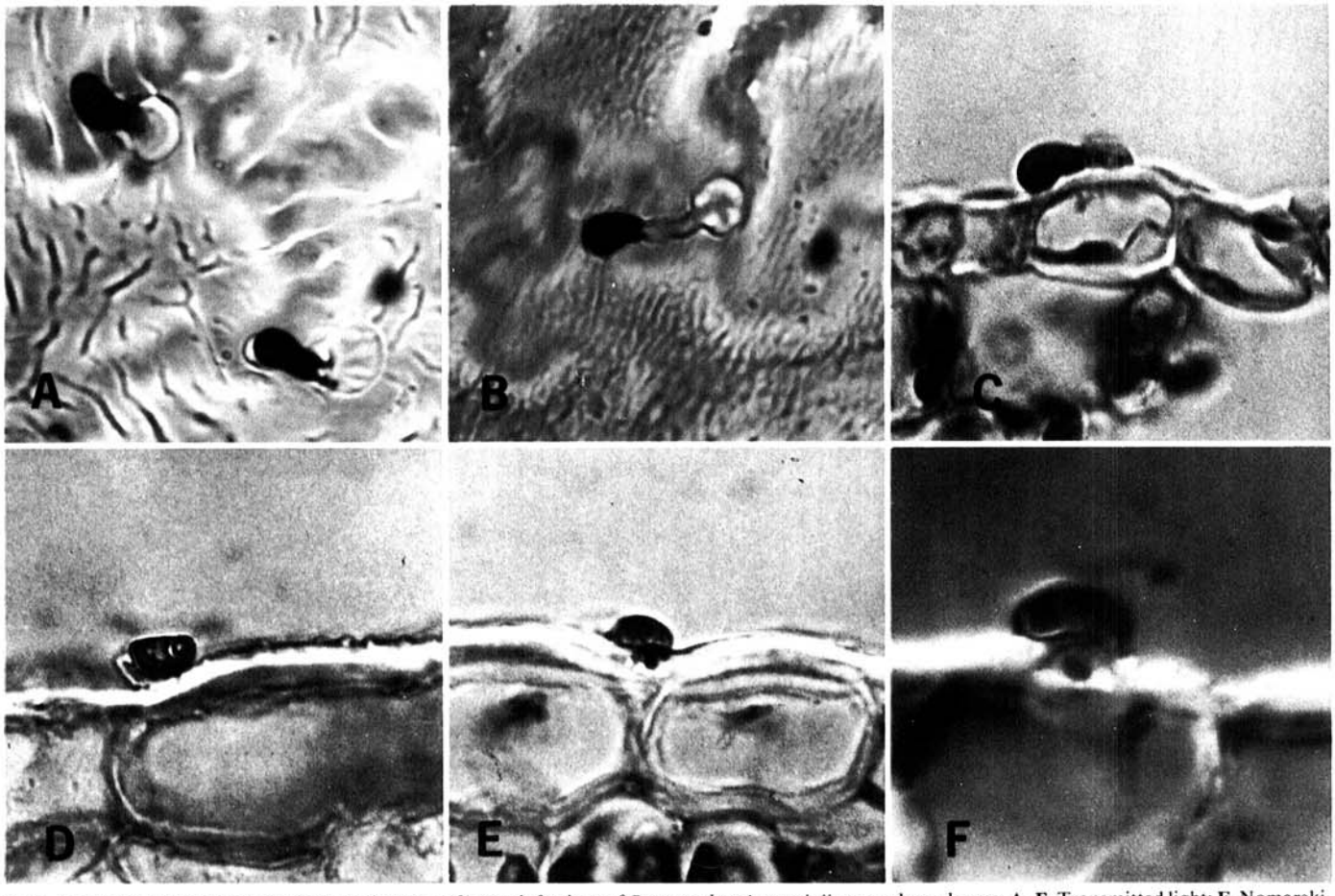

Fig. 2. Appressorial development and establishment of latent infections of *Botryosphaeria vaccinii* on cranberry leaves. **A–E,** Transmitted light; **F,** Nomarski optics; **A and B,** polystyrene leaf replicas; and **C–F,** sectioned material. **A,** Dark appressoria adjacent to hyaline spores on the adaxial surface of a cranberry leaf 24 hr after inoculation. **B,** Appressorium development with prior germ tube formation 24 hr after inoculation. **C,** Appressorium adjacent to a partially collapsed spore on the abaxial surface of a cranberry leaf 48 hr after inoculation. **D,** Appressorium and penetration peg on the adaxial surface of a cranberry leaf 72 hr after inoculation. **E,** Appressorium and infection hypha between the cuticle and epidermal cell wall 2 wk after inoculation. **F,** Appressorium and infection hypha within the cuticle adjacent to the epidermal cell wall 2 wk after inoculation. Note the change in the cell wall matrix surrounding the infection hyphae in **E** and **F.**

appeared less dense when examined with Nomarski optics (Fig. 2E,F). No further development was noted after 2 wk. Hyphal growth was typically limited to a small swelling, although occasional restricted lateral development was noted. No penetration of the epidermal cell wall was observed.

DISCUSSION

As previously suggested (5,12,17), *B. vaccinii* is capable of establishing latent infections on cranberry leaves. The present study shows that *B. vaccinii* can be isolated consistently from surface disinfested symptomless leaves 6 wk after infection under a range of environmental conditions. Microscopic examination demonstrated that spore germination, appressorial formation, and cuticular penetration can occur without subsequent intercellular or intracellular development.

The range of temperatures conducive to infection and establishment suggest that *B. vaccinii* can become established under a range of climatic conditions. We have isolated *B. vaccinii* from cranberry leaves collected in New Jersey, Massachusetts, Wisconsin, and Washington (17); areas that can vary markedly in climate. *B. vaccinii* was isolated by Friend (5) with increasing frequency from cranberry leaves collected in Wisconsin from June until November indicating that infection can take place throughout the growing season.

Latent leaf infections have an important role in the epidemiology of *B. vaccinii* on cranberry. We have observed pycnidia from April to October and pseudothecia from April to August on senescent leaves beneath healthy cranberry plants in Wisconsin and New Jersey (*unpublished*). Reproductive structures of *B. vaccinii*, however, are rarely produced on decayed fruit remaining from previous harvests, suggesting that sporulation on senescent leaves provides the major source of inoculum for fruit and leaf infections throughout the growing season. The role of latent leaf infections by *B. vaccinii* on cranberry is similar to that shown for *Guignardia citricarpa* Kiely on citrus (7,8). Both require sporulation on abscised leaves as the primary source of inoculum for fruit infections.

The histology of latent infections by *B. vaccinii* is similar to that shown for *G. citricarpa* on citrus (9) and *Colletotrichum musae* (Berk and Curt.) Arx on banana (*Musa*×*paradisiaca* L.) (4,10,14). Both develop subcuticular infection hyphae that remain dormant prior to resuming growth.

Muirhead (10) reported that dormant appressoria, rather than subcuticular hyphae, functioned as the latent structures of *C. musae* on banana. Continued development of infection hyphae was limited by a hypersensitive response in adjacent cells. However, we observed no cellular necrosis around infection hyphae of *B. vaccinii*.

Simmonds (14) reported an alteration in the cell wall material surrounding infection hyphae of *Colletotrichum* on mango (*Mangifera indica* L.), similar to that shown for *B. vaccinii*. The change in the cell wall matrix can be attributed to the production of extracellular degradative enzymes by the pathogen or to a host response to invasion, although Simmonds (14) also considered a host response to mechanical pressure as a possibility.

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