HortScience 17(6):951–953. 1982.

Mycorrhizal Distribution in Florida Rabbiteye Blueberries[1]

Lee A. Jacobs[2], Frederick S. Davies[2], and James M. Kimbrough[3]
University of Florida, Gainesville, FL 32611

Additional index words. *Elaphomyces persoonii*, ectendomycorrhizae, soil microbiology, *Vaccinium ashei*

Abstract. **Three rabbiteye blueberry (*Vaccinium ashei* Reade) plantations of different ages were surveyed in north Florida to determine the type and extent of mycorrhizal colonization. Ascocarps of an ectendomycorrhizal fungus, *Elaphomyces persoonii* Vitt., were found attached to the root of *V. fuscatum* Ait., a common wild blueberry. This fungus was identical morphologically to that isolated from roots of rabbiteye blueberry. Mycorrhizal colonization was greatest at the 8- and 18-year-old plantations, where at least 50% of the roots were colonized, and least at the 4-year-old planting and on 2-year-old bushes in the nursery. The greatest number of ascocarps was found in the wild near the most heavily colonized plantation. Soil P was highest at the 4-year-old and lowest at the 18-year-old planting. No monthly variation in the amount of mycorrhizal colonization was observed thoughout the year at the oldest site. Inoculum potential of ectendomycorrhizal fungi in the surrounding land, age of the planting, and P content of the soil appear to influence the extent of colonization of cultivated rabbiteye blueberry.**

Many *Vaccinium* species form endomycorrhizal associations in the wild which appear to be essential for survival (11). Coville (2), as early as 1910, observed mycorrhizae on all of the highbush blueberry species that he examined. Inoculation with mycorrhizal fungi prior to planting is being recommended as a means of increasing yields and survival of highbush blueberry in New Zealand (12).

However, not all *Vaccinium* species respond favorably to mycorrhizal colonization. Mowry and Camp (10) observed no relationship between vigor of rabbiteye blueberry and extent of mycorrhizal colonization. Similarly, Reich et al. (13) observed no growth response of highbush blueberries after they had been inoculated with mycorrhizal fungi. Furthermore, little is known about the fungal symbiont or the degree of colonization of rabbiteye cultivars in north central Florida.

Our objectives were to isolate and characterize the fungal symbiont and to determine the extent of colonization during an entire season for 3 rabbiteye blueberry plantations. We also measured soil P levels which are known to influence degree of mycorrhizal colonization for some plant species (4, 8).

Three rabbiteye blueberry plantations were selected in Alachua County, Fla., in 1979 that differed in age and past agricultural history. The Longnecker plantation was planted in 1961 on land formerly used to produce watermelons and is surrounded by pine-oak flatwoods. The University of Florida Horticultural Unit was planted in 1971 on very poorly drained flatwoods not previously used for agriculture. The entire planting is tile drained. The Snapp plantation was set out in 1975 onto cotton land that had been heavily limed for many years and is also bordered by pine-oak flatwoods. The soil type at each location is Leon fine sand which is a poorly drained soil usually associated with pine-oak flatwoods; soil pH averaged 4.5 at each location.

Roots of *V. fuscatum*, a native species which is known to be highly colonized by mycorrhizal fungi (5), were collected from the pine-oak flatwoods of Cross Creek, Fla. The degree of colonization of these roots was designated as the maximum obtainable for *Vaccinium* species in this area.

Mycelial isolates of the ericaceous endophyte from *V. fuscatum* and *V. ashei* were obtained using a direct-plating method (11). A section of mycorrhizal root was selected using a dissecting microscope. Mycorrhizal segments were excised, brushed clean, then placed in 30% hydrogen peroxide for 10 min. Segments were serially washed 3 times in distilled water, surface-sterilized with 6% sodium hypochlorite, and plated-out, 5 to a Petri dish, on a bacteriostatic 2% malt agar medium.

Each plantation was divided into quadrants, and 3 bushes were randomly sampled monthly from October 1979 to September 1980 to determine the extent of mycorrhizal colonization. Three root samples were taken per bush beneath the canopy with a soil auger at a depth of 3–8 cm. Seasonal variations in mycorrhizal colonization were also examined. Five plants at each location that were known to be mycorrhizal were repeatedly sampled monthly for 1 year. Repeated sampling did not damage the bush. No cultivar-related differences in colonization were observed; consequently, data from all cultivars were combined. Soil collected with the root samples was analyzed for P using the double-acid extraction method (15).

Roots were prepared and stained by a method modified from Ambler and Young (1). Main roots with attached feeder roots were placed in beakers, covered with a 10% KOH solution, and soaked overnight. Roots were then rinsed thoroughly and soaked in water for 1 hr. Any pigment remaining was removed by sample immersion in 10% hydrogen peroxide for 1 to 2 hr followed by rinsing in distilled water. Cleared roots were collected in a vial to which was added an equal volume of Trypan blue stain (100 ml lactic acid, 100 ml phenol, 200 ml glycerol, 200 ml distilled water, and 0.3 g Trypan blue stain).

Random samples of roots were removed from the stain and spread onto a slide. Twenty-five to 30 feeder roots (ca. 22 mm in length) were mounted under a 22-mm^2 cover slip. Mycorrhizal infection was estimated by determining the relative abundance of pelotons (cortical cells filled with hyphae) in each root sample. Three slides per replication were evaluated for mycorrhizal colonization by a visual estimation method adapted from Giovanetti and Mosse (3) (Table 1).

The morphology of mycorrhizae on *V. fuscatum* and *V. ashei* was identical. Positive evidence that the same fungus causes mycorrhizal colonization was established by the anastomoses of hyphae grown on the same Petri dish. Ascocarps which were found attached to the root of *V. fuscatum* were identified by J. M. Trappe, USDA Forest Service, Corvallis, Ore., as *Elaphomyces persoonii* (Fig. 1a). In addition to the ascocarps found attached to the root, other evidence included the similarity of hyphae attached to *E. persoonii* ascocarps with those forming mycorrhizae on *Vaccinium* roots.

Vaccinium fuscatum was found to be highly mycorrhizal in every sampling at Cross Creek. Mycorrhizae were formed on feeder roots that ranged in size from 40 to 120 μm in diameter. The finest of these hairlike roots had 4 cortical cells surrounding the stele; however, only the outer layer of these cells was colonized (Fig. 1b). Nonmycorrhizal roots in-

Table 1. Rating system used to determine the degree of mycorrhizal colonization for *V. ashei* and *V. fuscatum* roots.

Value	Criteria
8	Amount of colonization on *V. fuscatum* >6 roots/slide with pelotons
7	6 roots/slide with pelotons
6	5 roots/slide with pelotons
5	4 roots/slide with pelotons
4	2–3 roots/slide with pelotons
3	1 root/slide with pelotons
2	2 strands of hyphae/root
1	1 strand of hyphae/root

[1]Received for publication June 24, 1982. Florida Journal Series No. 3939.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[2]Fruit Crops Department.
[3]Botany Department.

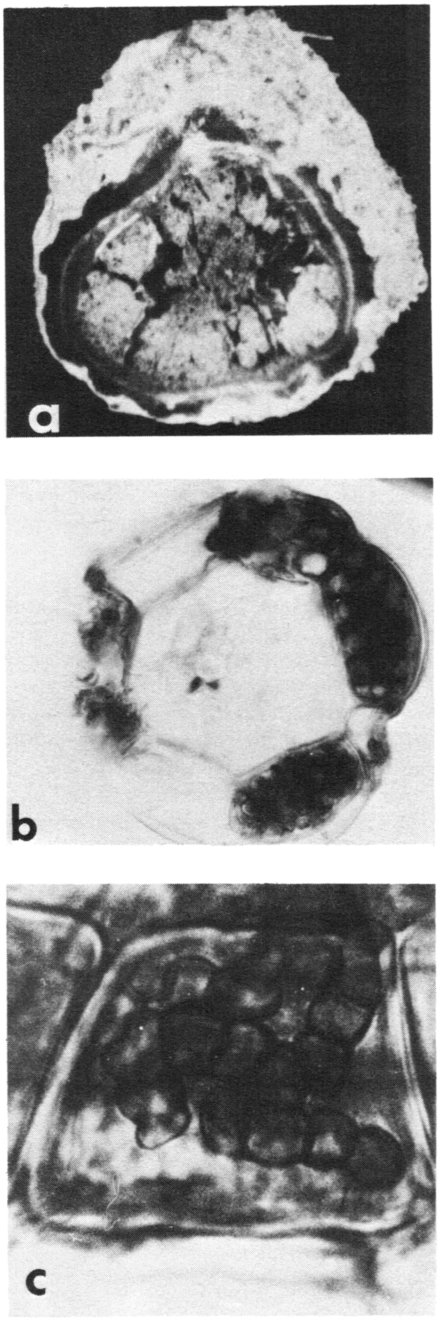

Fig. 1. *Elaphomyces persoonii*: a) Cross section of an ascocarp × 4 b) Cross section of cortical cells filled with hyphae, × 1300, c) Squash mount of a peloton, × 1650.

cluded young, rapidly growing roots and older, larger suberized roots. Colonized roots were distinguishable by the presence of pelotons (Fig. 1c).

Mycorrhizal colonization did not vary seasonally for randomly sampled plants within locations; therefore, data were combined and plant means determined for the entire year. The Longnecker plantation and the Horticultural Unit had the greatest extent of colonization, while the Snapp plantation was considered nonmycorrhizal (Table 2). Mycorrhizal colonization values ranged from 3 to 7 at Longnecker's, 2 to 5 at the Horticultural Unit, and 0 to 4 at Snapp's.

The yearly cumulative mean rating for randomly sampled plants at the Longnecker plantation, 5, and the Horticultural Unit, 4, are both within a range that is considered to be effectively mycorrhizal. Rhuele (14) considered ectomycorrhizal pine seedlings with 50% of their short roots colonized to be effectively mycorrhizal. This corresponds to a rating of 5 under the system used in this work. A rating of 1 at the Snapp plantation indicates the presence of fungal hyphae exterior to the root.

Levels of soil P also varied with location (Table 2). Soil P was highest at Snapp, followed by the Horticultural Unit, with the lowest levels at Longnecker.

The degree of mycorrhizal colonization did not vary significantly throughout the season in repeatedly sampled bushes at Longnecker, although means varied from 5 to 8 (Fig. 2). Jacobs et al. (5) found that less than 10% of rabbiteye blueberry roots became mycorrhizal after 6 months in pot culture. Apparently, even an entire season does not provide sufficient opportunity for measurable changes in colonization to occur.

Three factors appear important to the degree of mycorrhizal colonization in rabbiteye blueberry: 1) nature of the surrounding land; 2) age of the planting; and 3) P content of the soil. The Longnecker plantation is bordered on 2 sides by commercial pulpwood forests of *Pinus elliottii*. Over 50 ascocarps of *E. persoonii* were found within 20 m of the plantation. The Horticultural Unit has a strip of pine-oak forest protruding into it near the blueberry planting. No ascocarps of *E. persoonii* were found in the forest humus at this location during the survey. However, much of the forest adjacent to the planting had been recently converted to cropland; consequently, the inoculum potential of ectendomycorrhizal fungi for the land adjacent to this planting could not be determined. The Snapp plantation has a narrow strip of pine forest bordering one side. The other 3 sides are pasture and swamp. One ascocarp of *Elaphomyces granulatus* Fr. was found there during the survey. Pulpwood plantations surrounding the Longnecker plantation clearly offered an advantage over the other sites in ectendomycorrhizal inoculum potential.

A second possibly important characteristic is the age of the plantings. The Snapp and the Longnecker plantations had nursery blocks of 2-year-old plants that had been set out 1 year before this survey began. Root samples taken from these sites showed no mycorrhizal colonization. These blocks also lacked rodent tunnels and spores of *E. persoonii*. Rodents are a primary mode of spore dissemination in the wild (7). In contrast, root sampling commonly revealed rodent tunnels and spores of *E. persoonii* in older blocks at all 3 plantations and on *V. fuscatum* at Cross Creek. A considerable time may pass before field plantings are exposed to mycorrhizal inoculum. Highbush blueberry plants growing in New Zealand became 100% mycorrhizal only after 15 years when left to natural inoculation methods (12). It appears that a relatively high degree of mycorrhizal colonization at Longnecker's and the Horticultural Unit could be the effect of equilibration over time with ectendomycorrhizae adjacent to the rabbiteye plantation.

Table 2. Mycorrhizal colonization and soil P levels for 3 rabbiteye blueberry plantations.[z]

Location	Mycorrhizal colonization rating[y]	Soil P (ppm)
Longnecker	5a	16a
Hort. Unit	4a	38b
Snapp	1b	97c

[z]Values are means of 3 replications per location sampled monthly from October 1979 to September 1980, n = 36. Means followed by unlike letters are different based on Duncan's multiple range test, 5% level.

[y]O = no colonization; 8 = maximum colonization on roots of *V. fuscatum*.

A third factor which appears to be related to mycorrhizal distribution is the P content of the soil. High levels of P found at the Snapp plantation may be an important factor influencing mycorrhizal colonization at this site. Phosphorus concentration has been shown to have a direct effect on mycorrhizal fungi (4, 8). Inclusion of a 0.02 M phosphate buffer into the media inhibited growth of *Cenoccum geophilum* Fr. in culture (6). Similarly, 2 ericaceous endophytes, similar to those found on *V. ashei*, exhibited a lower growth rate when 550 ppm of P was included in the media (9). We also measured soil Ca^{+2}, NH_4^+, and NO_3^- but found P to be most consistently correlated with the degree of colonization. However, P level is a function of soil type and fertilizer practices and should not be considered as the limiting factor for mycorrhizal colonization.

Our data are in agreement with those of Mowry and Camp (9) who observed great differences in degree of colonization among cultivated rabbiteye blueberry bushes. Cultivated rabbiteye blueberry does not appear to be dependent on mycorrhizae for survival in north-central Florida. However, we do not know whether mycorrhizal inoculation of rabbiteye blueberries within a specific location would influence productivity as has been observed in New Zealand (12). Inoculum po-

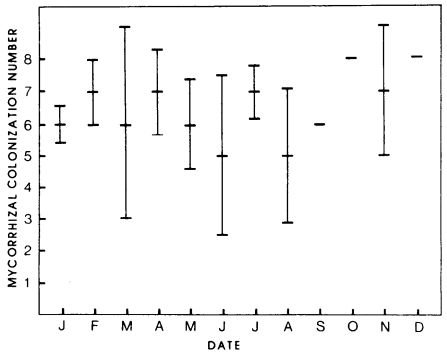

Fig. 2. Seasonal patterns of mycorrhizal colonization for *V. ashei* at the Longnecker plantation, 1979–80. Means of 5 bushes per date ± SD. (O = no colonization; 8 = maximum colonization on roots of *V. fuscatum*.)

tential of ectendomycorrhizal fungi in the surrounding land and age of the planting appear to be the most important factors in mycorrhizal colonization of rabbiteye blueberry in north-central Florida. However, cultural practices, soil type, and cropping history are also factors which may affect the extent of mycorrhizal colonization.

Literature Cited

1. Ambler, J. R. and J. L. Young. 1977. Techniques for determining root length infected by vesicular-arbuscular mycorrhizae. Soil Sci. Soc. Amer. J. 41:551–556.
2. Coville, F. V. 1910. Experiments in blueberry culture. U.S. Dept. Agr. Bureau Plant Indus. Bul. 193.
3. Giovanetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84:489–500.
4. Graham, J. H., R. T. Leonard, and J. A. Menge. 1981. Membrane mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. Plant Physiol. 68:548–552.
5. Jacobs, L. A., F. S. Davies, and J. W. Kimbrough. 1981. Mycorrhizal associations in wild and cultivated *Vaccinium* spp. in north central Florida. HortScience 16:145.(Abstr.)
6. Marx, D. H. and B. Zak. 1965. Effect of pH on mycorrhizal formation of slash pine in aseptic culture. For. Sci. 11:66–74.
7. Masser, C., J. M. Trappe, and R. A. Nussbaum. 1978. Fungal-small mammal interrelationships with emphasis on Oregon coniferous forests Ecology 59:799–809.
8. Menge, J. A., D. Steirle, D. J. Bagyaraj, E. L. V. Johnson, and R. T. Leonard. 1978. Phosphorus concentration in plant responsible for inhibition of mycorrhizal infection. New Phytol. 80:575–578.
9. Mitchell, D. T. and D. J. Read. 1981. Utilization of inorganic and organic phosphates by the mycorrhizal endophytes of *Vaccinium macrocarpon* and *Rhododendron ponticum* Trans. Brit. Myc. Soc. 76:255–260.
10. Mowry, H. and A. F. Camp. 1928. Blueberry culture in Florida. Fla. Agr. Expt. Sta. Bul. 194. p. 279–297.
11. Pearson, V. and D. J. Read. 1973. The biology of mycorrhiza in the Ericaceae. I. The isolation of the endophyte and the synthesis of mycorrhizas in aseptic culture. New Phytol. 72:371–381.
12. Powell, C. L. and P. M. Bates. 1981. Ericoid mycorrhizas stimulate fruit yield of blueberry. HortScience 16:655–656.
13. Reich, L., R. F. Korchak, and A. Thompson. 1981. The effects of certain edaphic factors on highbush blueberry (*Vaccinium corymbosum* L.) growth. HortScience 16:436. (Abstr.)
14. Ruehle, J. L. 1980. Inoculation of containerized loblolly pine seedlings with basidiospores of *Pisolithus tinctorius*. U.S. Dept. Agr. For. Ser. Res. Note SE-291.
15. Soil Science Research Report. 1979. University of Florida, Soil Science Dept. 79-1.

HortScience 17(6):953–954. 1982.

Gibberellic Acid-induced Fruiting of Lingonberries, *Vaccinium vitis-idaea* L. ssp. *minus* (Lodd.) Hult.[1]

Patricia S. Holloway[2] and Cecil Stushnoff[3]
Department of Horticultural Science and Landscape Architecture, University of Minnesota, St. Paul, MN 55108
David K. Wildung
North Central Experiment Station, University of Minnesota, Grand Rapids, MN 55744

Additional index words. cowberry, partridgeberry

Abstract. **A single application of 50, 100 or 500 mg/liter gibberellic acid (GA) during 75% full bloom induced seedless fruit development in lingonberries growing in their native habitat in Alaska. Fruit set was increased by the 500 ppm GA treatment in the absence of insect pollination. Fruit set was not increased by GA in open-pollinated plants. Berry weight and diameter were unaffected by GA treatments.**

Lingonberries are harvested commercially from their native habitat in Alaska, Canada, and throughout Eurasia. Low fruit set caused by inadequate pollination (4, 6), self pollination (4, 5), and cold temperatures during anthesis (9) limits marketable yields in some years. Fruit set ranging from 0 to 70% of blossom production has been reported (5, 6, 8, 18).

Fruit set has been increased in a diversity of crops by exogenous GA applications even under conditions of unfavorable weather and inadequate pollination (2, 17). Included in this group are *Vaccinium ashei* (16), *V. angustifolium* (1), *V. corymbosum* (7, 10, 12, 13, 14, 15), and *V. macrocarpon* (3, 11). In addition to increased fruit set, GA treatments have been found to decrease fruit size in *V. macrocarpon* (3, 11) and *V. ashei* (16). No experiments studying the response of GA applications on *V. vitis-idaea* have been reported. The purpose of this study was to elucidate the effects of GA on fruit set and fruit development in lingonberries.

In 1979, 4 blocks, 30 × 210 cm in size, were selected at random from a single, uniform population of lingonberries growing in a black spruce-birch forest near Fairbanks, Alaska. Each block was subdivided into four 30 × 30 cm treatment sections with 30 cm separating adjacent treatments. Thirty reproductive stems selected at random comprised each treatment unit. Aqueous solutions of GA (Pro-Gibb, Abbot Laboratories, North Chicago, Ill.) at 50, 100, or 500 mg/liter with 0.05% Tween 20 as a wetting agent were applied with a handsprayer until runoff at a rate of about 80 ml per m^2. Control plots were sprayed with a similar volume of water. The single application occurred on June 10 at 75% full bloom. The experiment consisted of a randomized complete block design with subsampling.

The 1980 experiment contained 4 randomized blocks and 8 treatments per block. A different population of lingonberries in the same locality comprised the experimental unit. GA was applied at the same rates as in 1979. The 30 stems in each treatment section either remained uncovered or were enclosed in individual glassine envelopes to prevent insect pollination. Stems were covered on May 28 prior to anthesis. The envelopes were removed for spray application on June 9 at 75% full bloom, immediately replaced, then permanently removed on June 26 following completion of petal fall.

In both 1979 and 1980, the number of flowers per stem was counted immediately prior to spray applications. Ripe fruit were counted, weighed, and the diameter was measured with calipers. Seed counts per berry and the percent seedless fruit were recorded. In 1979, fruit were harvested on Aug. 28, 30, and Sept. 1. Due to an extremely early snow fall on Sept. 2, 1980, fruit were harvested once on Sept. 5.

Fruit set percentages were not significantly influenced by GA treatments in the 1979 experiment (Table 1). The open-pollinated flowers set between 50 and 60% in all treatments. In 1980, similar results were observed for open-pollinated treatments, although the

[1]Received for publication Feb. 19, 1982. Paper number 12,071 of the Scientific Journal Series of the University of Minnesota Agricultural Experiment Station.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[2]Present address: SR-40581 Fairbanks, AK 99701.
[3]Present address: Department of Horticulture Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.