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Shoot Length Affects Susceptibility to Mummy Berry Blight within Highbush Blueberry Cultivars

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Abstract. **Shoot growth of six blight-resistant highbush blueberry (*Vaccinium corymbosum* L.) cultivars and of one susceptible cultivar was manipulated during the primary infection period of mummy berry disease to determine if some portion of the observed resistance was based on disease avoidance. In experiments across 2 years, resistant cultivars either increased continually in susceptibility or exhibited a peak and then decreased in susceptibility as shoots elongated. In a larger experiment that included both susceptible and resistant cultivars, peaks of susceptibility were identified for 'Bluejay', 'Darrow', and 'Jersey'. In contrast, general decreases in susceptibility were identified for 'Duke', 'Blueray', and 'Croatan' as shoots elongated. Shoot lengths associated with peak susceptibility varied among and within cultivars across experiments. The increases in susceptibility observed at longer shoot lengths were generally small. This finding suggests that cultivars identified as resistant have intrinsic levels of resistance, but maturity and general condition of the plant tissue can also affect disease levels.**

Mummy berry, caused by the fungus *Monilinia vaccinii-corymbosi* (Reade) Honey, is one of the most important fungal diseases of blueberry in North America because of its widespread occurrence and potential for reducing yield (Eck, 1988). The fungus overwinters on the soil surface as a pseudosclerotium (mummy). In the spring, pseudosclerotia germinate to form apothecia from which ascospores are produced. Ascospores initiate the spring cycle of mummy berry disease by infecting leaf, stem, and flower-bud tissue and inducing blight (Batra, 1983). Blighted tissue produces conidia that are carried by bees or wind to flower stigmas where they can germinate, grow down the style into the ovary, and produce mummified fruit to complete the life cycle.

Resistance to mummy berry disease in highbush blueberry cultivars offers an alternative to chemical control, which is presently dependent on two fungicides, *N,N'*- [1,4-piperazinediylbis (2,2,2-trichloroethylidene)] bisformamide (triforine) and methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate (benomyl). Resistant cultivars could complement proven sanitary and cultural control practices (Eck, 1988), making it possible to eliminate or reduce fungicide use. Several studies have documented various levels of resistance to *M. vaccinii-corymbosi* in highbush blueberry cultivars (Nelson and Bittenbender, 1971; Pepin and Toms, 1969; Stretch et al., 1995; Varney and Stretch, 1966). In an evaluation of 52 highbush blueberry cultivars, Stretch et al. (1995) reported consistent blight resistance for several cultivars, including 'Jersey', 'Elliott', 'Bluejay', 'Duke', 'Stanley', and 'Darrow'. Across 48 cultivars, Ehlenfeldt et al. (1996) found a highly significant correlation between blight incidence and average shoot length during the first week of ascospore release ($r = 0.74$, incorrectly reported originally as $r = 0.83$). Shoot lengths of the eight most susceptible cultivars were 32% to 91% greater than the average of all cultivars during the first week. In contrast, shoot lengths for the eight most resistant cultivars were 18% to 42% shorter than the average of all cultivars. These differences suggested that disease avoidance might be responsible for a measure of resistance to blighting in some cultivars (Burdon, 1987). Moreover, Lehman and Oudemans (1995, 1997a, 1997b) have recently demonstrated that populations of *M. vaccinii-corymbosi* have distinct developmental phenologies and ascospore release periods that appear to correspond to the development of the host. Noncoincident phenologies between host and pathogen may account for low incidence of shoot blighting observed for some cultivars. Our objective in this study was to manipulate shoot elongation in resistant and susceptible cultivars in order to evaluate the relationship between shoot length and susceptibility, and to determine whether blighting resistance of certain cultivars was due to avoidance.

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Materials and Methods

Experiment 1. The six most blight-resistant cultivars from a previous study, 'Jersey', 'Elliott', 'Bluejay', 'Duke', 'Stanley', and 'Darrow' (Stretch et al., 1995) were selected for evaluation. 'Bluehaven' was included as a blight-susceptible control. To produce plants with different shoot lengths in 1995, groups of five dormant, 2-year-old plants that had overwintered in cold frames were placed in an incubator for 1 or 2 weeks to induce vegetative shoot break and artificially advance shoot growth. Beginning on 16 Mar. 1995, a set of five potted plants (3-L pots) of each cultivar to be advanced two weeks (*adv+2*) were placed in an incubation chamber held at a constant 22 °C. The chamber was illuminated with a 400-W mercury vapor light suspended 1.5 m above the floor with a day length approximating natural conditions. On 23 Mar. a second set of plants to be advanced 1 week (*adv+1*) was added and incubation continued for another week. Two weeks after initiation, the temperature was lowered to 4 °C, and plants were held at this temperature until initial development of apothecial cups was observed in an outdoor inoculation block (10 Apr.). The two incubated groups were then placed outdoors with a third set of plants (*adv+0*) that had been subjected only to natural conditions. Plants were arranged in a completely random design of five replications, with individual plants as the replication unit. Plants were arranged in a 7 × 15 grid pattern with 0.45 m between plants. Inoculum pots, containing three to five apothecia each, were placed within and around the grid so that a source of spores was within 0.3 m of each plant. The pseudosclerotia for the inoculation pots were collected in late summer 1994 from the cultivar Weymouth grown near Chatsworth, N.J. Pseudosclerotia were placed on the surface of moist 1 washed sand : 1 peat moss (v/v) in 2-L pots and kept in an unheated cold frame to receive necessary chilling. In the spring, as apothecia started to develop, the pots were moved to the inoculation block. Shoot lengths were measured three times per week beginning on 11 Apr. and continuing to 27 Apr., when shoot blighting began to occur. This period represented the time of early vegetative budbreak of most cultivars under natural conditions through the period of sporulation of apothecia of *M. vaccinii-corymbosi*. Plants were evaluated by measuring five tagged shoots per plant from bud base to leaf tip, and calculating an average value for that plant. Efforts were made to tag five sequential shoots on a branch to get an accurate representation of both long terminal shoots and shorter subterminal shoots. In cases where shoots were broken or damaged during the course of the

Table 1. Experiment 1. Incidence (%) of shoot blight caused by *M. vaccinii-corymbosi* in relation to shoot length for seven highbush blueberry cultivars, and curvilinear for coefficients of determination. Shoot lengths were artificially advanced 1 to 4 weeks (*adv+1–4*).

Cultivar	Year	Blight (%)[z]					Shoot length (mm)[y]					Coefficient of determination
		adv+0	*adv+1*	*adv+2*	*adv+3*	*adv+4*	*adv+0*	*adv+1*	*adv+2*	*adv+3*	*adv+4*	(r^2)
Bluehaven	1995	92	97	42			16.6	22.3	32.3			0.93[ns]
	1996	82	---	42	22		5.1	---	30.6	42.2		
Bluejay	1995	7	33	42			3.8	14.0	25.1			1.00[***]
	1996	7	---	36	23		3.2	---	30.0	35.5		
Darrow	1995	10	19	16			4.6	10.3	20.6			0.16[ns]
	1996	2	---	12	17		6.6	---	27.5	61.4		
Duke	1995	10	54	34			6.5	22.3	39.9			0.97[ns]
	1996	3	---	25	4		9.0	---	40.1	66.6		
Elliott	1995	4	9	7			2.8	4.9	3.9			0.79[ns]
	1996	17	---	---	27	9	4.0	---	---	15.9	43.2	
Jersey	1995	22	15	31			2.5	6.6	12.8			0.91[ns]
	1996	19	---	33	54		4.6	---	29.0	67.0		
Stanley	1995	10	36	31			3.5	7.8	19.4			0.80[ns]
	1996	6	---	39	58		4.5	---	27.7	61.7		

[z]Average blight percentage for the five plants in each treatment.
[y]Five consecutive shoots per plant measured on each of five plants in treatment at start of infection period.
[ns, ***]Nonsignificant or significant at $P \leq 0.001$, respectively.

experiment, new shoots were selected and tagged. Shoot measurement values were averaged across cultivar and treatment for each date, and a weekly mean was calculated. Only means from the first week of ascospore release were used in calculations. Blight evaluation was done using the methodology described by Stretch et al. (1995). Blighted shoots were removed and tallied three times weekly from 29 Apr. to 15 May, after which the remaining unblighted shoots were tallied.

The experiment was repeated in 1996, but incubation times began earlier and were extended to 2 and 3 weeks (*adv+2* and *adv+3*, respectively) for 'Jersey', 'Bluejay', 'Duke', 'Stanley', 'Darrow', and 'Bluehaven' to further increase shoot length. 'Elliott', which was exceptionally slow to break in 1995, was incubated for 3 and 4 weeks (*adv+3* and *adv+4*, respectively). These plants were treated similarly to those above and placed outdoors on 13 Apr. 1996. Shoot lengths were evaluated on 15, 17, and 19 Apr., and blighting assessed from 3 May through 17 May. The inoculum for this study was produced from pseudosclerotia collected in late summer 1995 from various cultivars growing on the Rutgers Blueberry and Cranberry Research Station, Chatsworth, N.J.

Experiment 2. A larger experiment was also designed in 1996, using ≈50 plants each of the resistant cultivars Bluejay, Darrow, Duke, Jersey, and Stanley, as well as the highly susceptible cultivars Blueray and Croatan. From 7 Feb. to 12 Mar., six plants per week (≈1 plant per day) were moved from unheated cold-frames to a greenhouse heated to 21 °C. On 13 Mar., all plants were moved outdoors to an inoculation block adjacent to that previously described and randomized within cultivars. Spacing for plants and inoculum pots was identical to that previously described. The average shoot length for each plant was determined a single time on 15 Apr. 1996, as previously described, and blight evaluated on 30 Apr., 6 May, and 15 May. Unblighted shoots and any remaining blighted shoots were counted on 20 May.

Data analysis. To determine maximum susceptibility, and shoot length corresponding to maximum susceptibility, regression curves were calculated using a Lorentzian peak regression equation of the form $y = a + b/[1 + \{(x - c)/d)\}^2]$ (Jandel Scientific Tablecurve software, ver. 3.11, San Rafael, Calif.). In this equation, the c coefficient yields the maximum blight (y) for positive shoot lengths (x). In Expt. 1, regressions were evaluated on combined means from 1995 and 1996. For Expt. 2, values more than 2.5 standard deviations from the cultivar blight mean were discarded as outliers. All subsequent calculations were done on the remaining values, allowing a more conservative evaluation of any true peak, since all discarded outliers represented abnormally high values. No more than two data points were discarded for any group.

Results and Discussion

In 1995 average blight susceptibility increased from the *adv+0* group to the *adv+1* group in 6 of the 7 cultivars (Table 1). Among these two groups, only 'Jersey' failed to exhibit an increase. Across the three advancement groups, susceptibility of 'Bluehaven' and 'Duke' was maximum at *adv+1*, then declined (≥20 percentage points) at *adv+2*, while changes in other cultivars were minor. In 1996, when plants were advanced for greater periods of time, susceptibility declined between the *adv+2* and *adv+3* groups for 'Bluehaven', 'Bluejay', and 'Duke', but increased in 'Darrow', 'Jersey', and 'Stanley'. A decrease occurred between *adv+3* and *adv+4* for 'Elliott'. Since corresponding treatments from the two years exhibited generally similar levels of blighting, data were combined for regression analysis. Curvilinear regressions utilizing a Lorentzian peak equation showed high coefficients of determination (r^2), but only 'Bluejay' had a significant fit. The regression for 'Bluejay' had a peak susceptibility of 43.1% at a shoot length of 22.7 mm. The r^2 values for the other cultivars ranged from 0.16 to 0.97, but the small number of points (6) used resulted in nonsignificance.

Similar analyses of the data from Expt. 2 produced significant regressions for all cultivars except 'Stanley' (Fig. 1). The susceptible cultivars Blueray and Croatan both exhibited high susceptibility at short shoot lengths, followed by decreasing susceptibility as shoot lengths increased (Fig. 1 a and b). 'Blueray' exhibited a nominal peak at a shoot length of 3.7 mm; however, this value may be regarded as essentially zero, since unexpanded buds measured 2 to 3 mm. At the regression maximum, 60.2% blight was predicted. 'Croatan' was similar, but exhibited no peak at positive shoot lengths, only a steady decline in susceptibility (Fig. 1b). Its maximum regression value of 42.0% occurred at the y-intercept. 'Croatan' exhibited more variability for blighting at short shoot lengths than did 'Blueray'. These low values decreased the mean blight value for 'Croatan' and resulted in a lower r^2. The range of values for 'Croatan', however, was nearly identical to that of 'Blueray'. The resistant cultivars presented a mixed picture in their regression plots. 'Duke' was most like the susceptible cultivars, in that it exhibited a continuous decrease in susceptibility as shoot length increased (Fig. 1c), although its relative levels of blight were much lower than those of the susceptible cultivars. 'Duke' exhibited a blight regression maximum of 19.0% at the y-intercept; however, the actual blight values averaged 6.8% across shoot lengths less than 25 mm, and 0.5% across all greater lengths. 'Bluejay' and 'Jersey' showed maximum blight at shoot lengths of 15.5 and 8.6 mm, respectively, which corresponded to regression maxima of 9.1% and 9.9% blight, respectively (Fig. 1 d and e). The regression for 'Darrow' exhibited a peak corresponding to 15.4% blight at a shoot length of 34.6 mm; however, this peak was unlike the other regressions in that its maxima exceeded the highest data value used to produce it (Fig. 1f). This peak was also unlike the other peaks in the steepness of its rise and fall. The shoot length range of 0 to 25 mm averaged 3.4% blight, 25 to 50 mm (the range incorporating the peak) 1.5% blight, and the region from 50 through 175 mm, 0.6% blight. Thus, although the regression was significant, the applicability of its results for

comparisons with other cultivars must be questioned. Apart from these considerations, 'Darrow' appeared to have the consistently lowest susceptibility (greatest resistance) among the cultivars tested. 'Stanley' had a nonsignificant regression, but showed values suggestive of steady or slightly decreasing susceptibility, and overall low values (Fig. 1g).

Among the resistant cultivars in Expt. 2, the highest blight regression maximum for a well-formed peak (i.e., excluding 'Darrow') was 9.9% for 'Jersey'. This finding suggests that while susceptibility increases with shoot length, these increases are not large, and that a substantial portion of the observed resistance in these cultivars is due to factors other than avoidance. The susceptibility of 'Duke' decreased continuously. 'Stanley', which appeared ambiguous in Expt. 1, also showed no defined peak in this experiment, but showed a relatively constant resistance response. The shoot lengths at the regression maxima where good peaks existed were 15.5 and 8.6 mm for 'Bluejay' and 'Jersey', respectively. The maximum for 'Duke' was not a true peak and was essentially the same as the dormant vegetative shoot length (2 to 3 mm).

Several interesting observations can be made from these data. A shift in susceptibility maxima occurred between Expts. 1 and 2 for 'Bluejay' and 'Duke', and a change in trend occurred between Expts. 1 and 2 (increasing vs. peaking) for 'Jersey'. We believe the reason for this discrepancy is the relative tenderness of the tissue involved. The plants in Expt. 1 were subjected to several weeks at high humidity in an incubation chamber, resulting in relatively soft, tender tissue. The materials in Expt. 2 were accelerated in a heated greenhouse at much lower humidity levels, resulting in more hardened tissue. Hildebrand and Braun (1991) noted increased susceptibility in tissue subjected to freezing damage. Tender tissue from incubator-grown plants presumably was more susceptible to tissue damage when transferred to cool outdoor conditions. We believe that the plants from the greenhouse might have more closely represented plants under natural conditions, but their true condition probably lay somewhere in between. Another observation is that many plants with apparently optimal shoot lengths for infection, in both resistant and susceptible cultivars, developed no blight. This was not the result of localized lack of inoculum, since susceptible plants from this test and several adjacent tests all showed extremely high levels of blight. This lack of blighting in some plants may reflect the relative condition of the tissue and the still largely unknown critical conditions for optimal infection.

Since we could not determine any single shoot length value at which maximum susceptibility occurred, screening under natural conditions still appears to be the best option unless large numbers of plants are available for manipulation of shoot length. For the cultivars we selected, the regressions suggested that increases in susceptibility with increasing shoot length were not inordinately large, and that these plants had both avoidance and biochemical resistance. Note that in our original screenings to identify sources of resistance, considerable efforts were made to ensure that inoculum was present as long as possible during the trial. This should guarantee that some biochemical resistance would be found among cultivars identified as most resistant, since the long inoculum period would largely overcome avoidance. 'Darrow', which was evaluated in both studies, appeared to have biochemical resistance as well as avoidance. 'Elliott' broke bud so slowly that avoidance alone might be a sufficient mechanism to assure functional resistance, yet it also exhibited substantial resistance at all shoot lengths. All of the highly resistant cultivars identified in our original screening appear suitable as breeding parents, which suggests that large populations of progeny can be derived with good resistance and good horticultural quality.

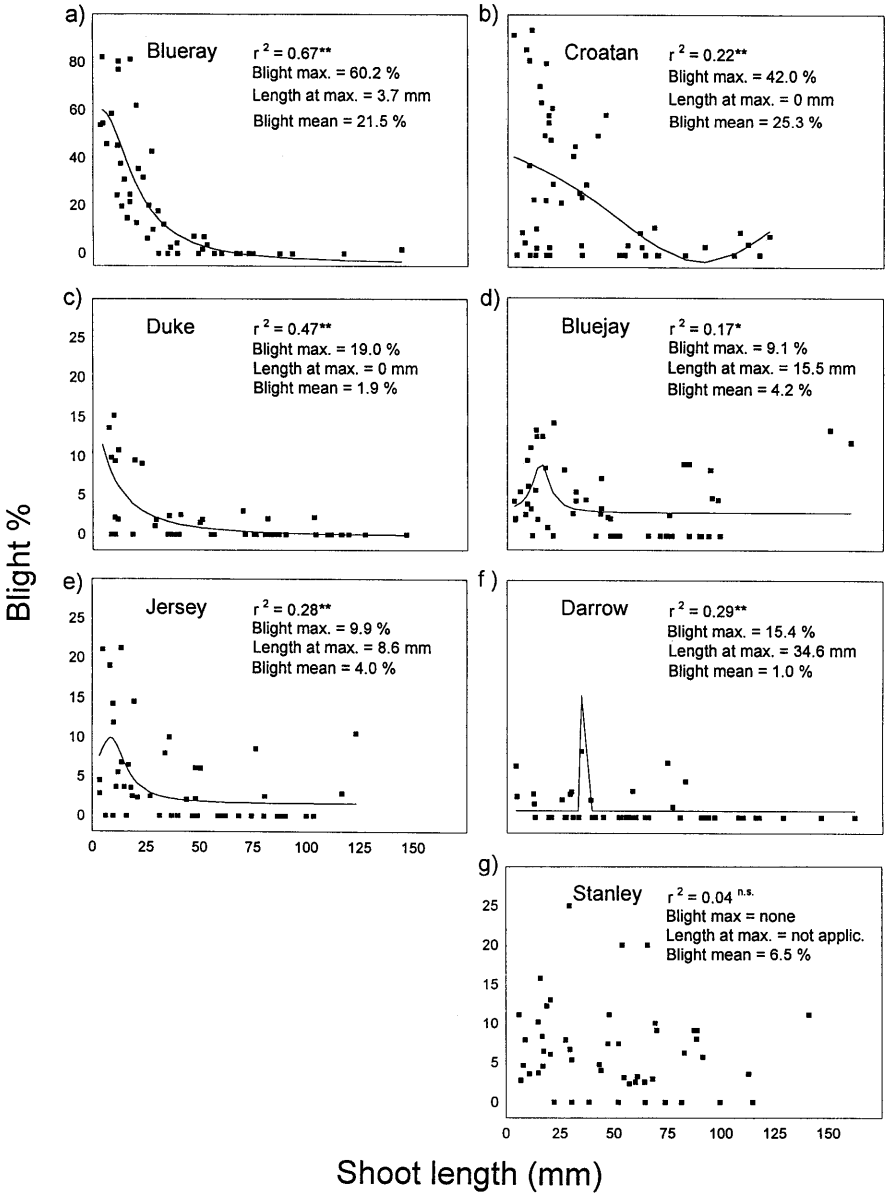

Fig. 1. (**a–g**) Experiment 2. Lorentzian peak regressions of shoot blight incidence caused by *M. vaccinii-corymbosi* vs. shoot length in highbush blueberry plants with artificially advanced foliar growth. Note that scale of abscissa for susceptible cultivars Blueray and Croatan is greater than that for the resistant cultivars Duke, Bluejay, Jersey, Darrow, and Stanley. Coefficients of determination (r^2) nonsignificant (NS) or significant at $P < 0.05$ (*), 0.01 (**), or 0.001 (***). Regressions of 'Croatan' and 'Duke' had no peaks at positive shoot length values; blight maxima are values at y-intercept (i.e., shoot length = 0 mm).

Literature Cited

Batra, L.R. 1983. *Monilinia vaccinii-corymbosi* (Sclerotiniaceae): Its biology on blueberry and comparison with related species. Mycologia 75:131–152.

Burdon, J.J. 1987. Diseases and plant population biology. Cambridge Univ. Press, Cambridge, England.

Eck, P. 1988. Blueberry science. Rutgers Univ. Press, New Brunswick, N.J.

Ehlenfeldt, M.K., A.W. Stretch, and V. Brewster. 1996. Genetic and morphological factors affecting mummy berry blight resistance in highbush blueberry cultivars. HortScience 31:252–254.

Hildebrand, P.D. and P.G. Braun. 1991. Factors

affecting infection of lowbush blueberry by ascospores of *Monilinia vaccinii-corymbosi*. Can. J. Plant Pathol. 13:232–240.

Lehman, J.S. and P.V. Oudemans. 1995. Differences in rates of apothecium development between populations of *Monilinia vaccinii-corymbosi*. Phytopathology 85:1133. (Abstr.)

Lehman, J.S. and P.V. Oudemans. 1997a. Phenology of apothecium production in populations of *Monilinia vaccinii-corymbosi* from early- and late-maturing blueberry cultivars. Phytopathology 87:218–223.

Lehman, J.S. and P.V. Oudemans. 1997b. Phenology of the mummy berry fungus and the blueberry host: Implications for resistance breeding. Acta Hort. (In press.)

Nelson, J. and H.C. Bittenbender. 1971. Mummy berry disease occurrence in a blueberry selection test planting. Plant Dis. Rpt. 55:651–653.

Pepin, H.S. and H.N.W. Toms. 1969. Susceptibility of highbush blueberry varieties to *Monilinia vaccinii-corymbosi*. Phytopathology 59:1876–1878.

Stretch, A.W., M.K. Ehlenfeldt, and V. Brewster. 1995. Mummy berry blight resistance in highbush blueberry cultivars. HortScience 30:589–591.

Varney, E.H. and A.W. Stretch. 1966. Diseases and their control, p. 236–279. In: P. Eck and N.F. Childers (eds.). Blueberry culture. Rutgers Univ. Press, New Brunswick, N.J.