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SCREENING Highbush BLUEBERRY
CULTIVARS FOR RESISTANCE
TO PHOMOPSIS CANKER

presented by

Jean Beard Baker

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~~Master of Science~~ degree in ~~Horticulture~~

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SCREENING Highbush blueberry
cultivars for resistance
to Phomopsis canker

by

Jean Beard Baker

A thesis

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

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ABSTRACT

SCREENING Highbush BLUEBERRY CULTIVARS FOR RESISTANCE TO PHOMOPSIS CANKER

by

Jean Beard Baker

Stem flap, stem freeze, needle pierce and leaf tear wounding techniques were examined for inoculation procedures of one-year-old rooted microshoots and two-year-old rooted hardwood cuttings. The needle pierce was chosen for screening highbush blueberry cultivars.

Microshoots and hardwood cuttings of nine highbush blueberry cultivars were placed in a factorial experiment to compare their resistance to *Phomopsis* canker. Shoot deaths from inoculated plants were recorded. Resistance was quantified by calculating a rate of mortality, length of survival and percent surviving for every cultivar and age class. Fungal reisolations from each cultivar of every block demonstrated that *Phomopsis vaccinii* caused shoot death.

Stem flap and needle pierce are effective inoculation techniques, but are too labor intensive for regular screening of high numbers of plants. 'Elliot' and 'Bluetta' demonstrated high levels of resistance with low rates of mortality, high lengths of survival and the highest percentages of surviving shoots.

**This thesis is dedicated in memory of my parents,
Joseph Edgar and Joyce Myers Beard**

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INTRODUCTION

Phomopsis canker is a serious fungal disease of highbush blueberries (*Vaccinium corymbosum* L.) in the southern regions of Michigan. The causal agent, *Phomopsis vaccinii* Shear, infects blueberry canes in Michigan by entering through an injury. The pathogen induces cane dieback which may lead to the death of the entire bush. Phomopsis canker is one of the primary factors limiting Michigan's highbush blueberry production (Hancock and Draper, 1989; Hanson and Hancock, 1990; Ramsdell, 1992). Therefore, locating a source of resistance would greatly benefit the future of this industry.

Early Research on *Phomopsis vaccinii*

Early research attempted to define the symptoms of Phomopsis canker. The pathogen was described as a fungal disease of cranberries (*Vaccinium macrocarpon*) by C. L. Shear in 1931. In 1936, Marguerite Wilcox reported that *Phomopsis vaccinii* had been isolated from blueberries in New Jersey, Massachusetts and North Carolina. Investigations continued that associated *Phomopsis vaccinii* with the formation of

blueberry stem galls (Brown, 1938). Further research defined the pathogen as a cause of twig blight that was distinct from the stem gall reported by Brown (Wilcox, 1939 and 1940). Studies on *Phomopsis vaccinii* were not conducted for many years and the pathogen was only described as a blight affecting blueberry stems (Demaree and Wilcox, 1947; Childers, 1949; Goheen, 1953; Ranieri, 1961; Varney and Stretch, 1966).

In 1974, Weingartner and Klos utilized field observations and Koch's postulates to determine the causes of highbush blueberry canker and dieback diseases in Michigan. They demonstrated that *Phomopsis vaccinii* forms cankers on two-year-old wood of blueberries. These findings were in contradiction to Wilcox's research in 1939 stating that *Phomopsis* causes only localized lesions on succulent stem tissue. They also reestablished that *Phomopsis vaccinii* does not produce stem galls. Weingartner and Klos (1974) suggested the terms "Phomopsis canker and dieback" to describe the symptoms of the disease in Michigan. These terms are now widely accepted.

Characteristics of *Phomopsis vaccinii*

Classification of Causal Agent

Currently *Phomopsis* is categorized in the fungal class,

Coelomycetes and grouped with other fungi that produce asexual spores in pycnidia in the order Sphaeropsidales (Agrios, 1988). Shear originally specified the fungus to be *Diaporthe vaccinii* as it appeared on the fruit and stems of cranberries (Shear, Stevens and Bain, 1931). Wilcox later determined that *Phomopsis vaccinii*, the asexual stage of *Diaporthe vaccinii*, was the pathogen isolated from blueberries (Wilcox, 1939). *Diaporthe vaccinii* has been cultured on artificial media, but it has never been observed on field-grown blueberry bushes (Wilcox, 1940; Weingartner and Klos, 1975).

Pathogenicity of Disease

The first reports from inoculation experiments indicated that *Phomopsis* infects succulent shoots or leaves through natural openings (Wilcox, 1939). These experiments used spore suspensions collected from blighted blueberry shoots and rotten cranberry fruit in Massachusetts. Wilcox (1939) discovered that either wounded or non-wounded plants would develop lesions and that the infection would eventually spread and girdle older, woody stems. This research was later replicated in North Carolina using two different *Phomopsis vaccinii* isolates from North Carolina and Michigan (Milholland, 1982). Twig blight symptoms appeared on two-year-old bushes of the cultivar 'Murphy', 2 weeks after the

Phomopsis isolates were sprayed onto terminal flower buds.

Several studies have shown that in Michigan, *Phomopsis vaccinii* requires an injury for infection. When healthy, non-wounded bushes were placed under heavily diseased, field plantings for 1-month intervals throughout the growing season, no infection was detected (Parker and Ramsdell, 1977). Experiments in Michigan indicated that non-wounded bushes were not infected by inoculations with conidial suspensions or mycelial blocks placed on the stems (Parker and Ramsdell, 1977). Blueberry stems that were wounded by mechanical means or freezing prior to inoculations became infected. This indicates that an injury to the plant by mechanical means is required for infection in Michigan.

Characteristic Symptoms

The most obvious symptom of infection by *Phomopsis vaccinii* is flagged or wilted stems on field plantings of blueberries during hot weather (Varney and Stretch, 1966; Weingartner and Klos, 1975; Ramsdell, 1992). It has been reported that wilted stems first appear during July and August and may continue to appear until October (Weingartner and Klos, 1975). Hyphal growth of the fungus through the intercellular spaces of the stem cortex causes a host plant to form tyloses which may clog the vascular system (Daykin and

Milholland, 1990). Shriveled, reddish leaves remain on necrotic or infected shoots. Discolored xylem has often been observed in infected stems. This is also a common symptom of cold injury (Varney and Stretch, 1966; Weingartner and Klos, 1975).

Diseased tissue can appear brown or black with a definite demarcation line appearing between the infected portion and the green, healthy shoots or canes. As the disease progresses, long and narrow cankers covered with bark or epidermis often form on woody stems (Weingartner and Klos, 1975). Black pycnidia may grow within the cankers or on diseased stems. Pycnidia have been found on leaves and over-wintering twigs (Wilcox, 1939). Isolations from diseased stems grown on artificial media is the best method to positively distinguish *Phomopsis* canker from other diseases or environmental conditions that may cause cane dieback in blueberries (Weingartner and Klos, 1975; Parker, 1976).

Period and Mode of Infection

Investigations have not determined the exact period of infection in the field, but the period of *Phomopsis vaccinii* spore dispersal has been identified (Parker and Ramsdell, 1977). Research showed that the majority of fungal spores could be trapped during periods of rain from pre-bloom to late

July in Michigan (Parker and Ramsdell, 1977). "Spores ooze from pycnidia during wet or humid periods and are spread by washing, splashing and windblown raindrops." (Ramsdell, 1992). Optimal mycelial growth and spore germination on artificial media has been obtained at temperatures from 21-27°C (Wilcox, 1939; Parker and Ramsdell, 1977). These results demonstrate that colder temperatures most likely limit the spread of *Phomopsis* to northern regions of Michigan (Weingartner and Klos, 1975).

Histopathological research has shown that *Phomopsis vaccinii* colonizes blueberry stems by hyphal growth in intercellular and intracellular spaces of the cortex (Daykin and Milholland, 1990). Daykin and Milholland discovered that tyloses often formed in the green, uninfected part of the stem in advance of the pathogen's hyphal growth. The pathogen continues its hyphal growth from the infected cortex laterally into the xylem and phloem tissues. If the cortex is completely colonized by the pathogen, the hyphae may invade the pith of the stem. Although the causal organism of *Phomopsis* canker does not require a wound for infection in North Carolina, these studies most likely depict the pathogenicity of *Phomopsis vaccinii* in Michigan.

Control Strategies

At present, there are only moderately effective chemical

controls for this disease. Research using Difolatan and Benlate sprays at different intervals during the growing season yielded only a 36%-58% decrease in the spread of Phomopsis canker (Parker and Ramsdell, 1977). Applications of protectant fungicides are not very effective because Phomopsis canker is a systemic disease. Once the fungal infection has grown into the crown of the bush, new shoots originating from the crown become diseased and die (Parker and Ramsdell, 1977).

Cultural controls combined with fungicide applications are currently the best way to limit the spread of this pathogen. Plants need to be maintained in a healthy and vigorous condition so that they are less susceptible to infection. Planting in areas of frost pockets or spring frosts should be avoided since cold injury predisposes plants to infection (Weingartner and Klos, 1975; Ranieri, 1961; Ramsdell, 1992). Weak canes should be annually pruned from bushes. It is also recommended that diseased stems be pruned as close to the crown as possible (Demaree and Wilcox, 1947; Childers, 1949; Ramsdell, 1992).

Cultural controls are not totally effective in preventing the spread of Phomopsis canker. The majority of blueberries in Michigan are mechanically harvested. Stems can be severely damaged when fruit is shaken or beaten from the bushes predisposing the plant to a *Phomopsis vaccinii* infection.

Heavily diseased bushes are usually removed from the field, but this is a costly and time-consuming procedure. It takes up to 6-10 years before highbush blueberries are into full berry production (Brun, 1992). Removing and replacing diseased plants is not a practical solution for producers.

Research Goals

An appropriate way to control the spread of Phomopsis canker would be to locate a source of resistance within highbush blueberry cultivars. The first goal of this research was to test different inoculation techniques for screening blueberries. Experiments were set up to determine which technique required the least amount of time and labor to perform. Tests were also structured to develop an inoculation technique that could be used on one-year-old rooted cuttings. This would allow a researcher to use greenhouse space as efficiently as possible. After the development of an effective screening procedure, the second goal was to test the most popular highbush blueberry cultivars for resistance to Phomopsis canker.

MATERIALS AND METHODS

General Plant Culture

One-year-old rooted microshoots and two-year-old, rooted hardwood cuttings of 'Bluecrop', 'Bluejay', 'Blueray', 'Bluetta', 'Elliot', 'Jersey', 'Patriot', 'Rancocas' and 'Spartan' cultivars were obtained from DeGrandchamp's Nursery in South Haven, Michigan. One-year-old 'Rancocas' rooted microshoots were produced at Michigan State University by tissue culture and rooted in the Plant Science greenhouses in East Lansing, Michigan.

Microshoots were removed from magentas (tissue culture boxes) and separated from adjoining shoots. Two hundred shoots per flat were placed in Bacto Pro Plant soil mixture (Michigan Peat Company, Houston, TX) and covered with a clear plastic humidity dome. Flats were placed under greenhouse benches for 3 to 4 weeks and the humidity dome was slowly tipped opened to acclimate plants to ambient humidity. The rooted microshoots were then transferred to 48-section cell packs in Bacto Pro Plant Mix.

Commercial producers generally root hardwood cuttings in

covered wooden frames of varying sizes in greenhouses (Schulte and Hancock, 1983). Shoots from the previous season's growth are obtained from the field in winter or early spring and usually placed into peat mixtures. Cuttings take from 3-4 months to root and then are planted approximately 45 cm apart in nursery rows.

Two-year-old plants were potted into Bacto Pro Plant Mix (Michigan Peat Company, Houston, TX) in 7 1/2 inch (18.75 cm x 13.97 cm) plastic pots. One-year-old plants were also potted into a Bacto Pro Plant soil mixture using 4 1/2 inch (11.25 cm x 8.89 cm) plastic pots. The plants were watered daily and fertilized once a week with a solution of Peter's 20-20-20 fertilizer (Grace Sierra, Fogelsville, PA). Phosphoric acid was used to adjust the pH of the fertilizer to 5.0. All plants were maintained in the Plant Science greenhouses for at least two weeks before the different treatments were initiated. All of the following research was conducted in the Plant Science Greenhouses on the campus of Michigan State University.

Fungus Isolation

A culture of *Phomopsis vaccinii* was obtained from Dr. Donald Ramsdell, Dept. of Botany and Plant Pathology, Michigan State University. The fungal culture was isolated in 1976

from a blueberry stem canker on a diseased 'Berkeley' bush on the Weipert Farm in West Olive, Michigan. The isolate was grown on Difco Potato Dextrose Agar (PDA) in a petri dish and stored at 4°C.

For all inoculation experiments, subcultures were obtained from the stem canker isolate and grown on PDA. Difco PDA was prepared according to label instructions. The dehydrated PDA powder was put into solution and melted by heating it for five minutes in an autoclave at 121°C. The solution was stirred and returned to the autoclave to heat sterilize for fifteen minutes at 121°C. The solution was poured into petri dishes to an approximate depth of 0.50 cm under a Laminar flow hood and allowed to cool.

Two black pycnidia from the stem canker isolate were placed into each petri dish under the Laminar flow hood with a sterile metal spatula. Subcultures were grown under cool, white florescent lamps at a photosynthetic photon flux (PPF) of 50 micromole/m²/sec. Subcultures were placed in a refrigerator at 4°C when white mycelium covered the entire plate surface and black pycnidia were present. During the course of research, isolates were subcultured every 30 days to retain fresh fungal cultures. Difco PDA was also poured into petri dishes at an approximate depth of 0.50 cm and stored in a refrigerator at 4°C to be used for control (mock) inoculations.

Research to Develop Inoculation Techniques

Experiments on One-year-old Rooted Microshoots

The first study utilizing one-year-old plants was conducted with 40 'Rancocas' rooted microshoots. The plants varied in shoot length from 5-10 cm and were approximately 0.65 cm in circumference. A specific treatment was applied to one shoot per plant. Ten plants were used for each treatment.

A comparison of two inoculation techniques was performed using a completely randomized design with four different treatments; 1) Shoots were pierced with a sterile Becton-Dickenson No. 5175 needle on a 10 ml syringe, 2) One leaf was torn from stems at the point where the petiole joins the shoot. The shoots from treatments 1 and 2 were inoculated at the sites of injury with 1-2 pycnidia and associated mycelium from *Phomopsis vaccinii* subcultures using a sterilized, metal spatula. The mechanical wounding techniques used in treatments 1 and 2 were repeated for treatments 3 and 4, but inoculations made at the sites of injury contained only PDA. Treatments 3 and 4 were observed as controls. Observation of these mock inoculations was important to prove that the plants did not die from the applications of PDA to the wounded tissue or from the injury itself.

The points of injury and inoculation were wrapped with

moistened Kim wipes (Kimberly Clark). Parafilm (American Can Co., Greenwich, CT.) was used to secure the wraps to the blueberry shoots and to retain moisture for effective fungal growth. Plants were observed each day and the number of shoots that had died from the point of inoculation to the tip of the shoot per treatment were counted.

A second experiment was performed to further examine the effectiveness of inoculation techniques on rooted microshoots and to compare different wounding techniques. Plants were placed in a randomized complete block design with three replications per treatment. This study utilized 144 'Rancocas' blueberries with 48 plants per block and eight plants per treatment.

One of six different treatments was applied to one shoot from each blueberry plant; 1) Shoots were injured with a sterilized, Bard-Parker No. 11, pointed scalpel blade. An approximate 0.32 cm flap was cut downward with the blade at an internode on a shoot, 2) A sterile Becton-Dickenson No. 5175 needle on a 10 ml syringe was used to pierce the shoots. 3) A single leaf was torn from a stem as previously described. Shoots from treatments 1, 2 and 3 were inoculated with 1-2 pycnidia and associated mycelium using a sterile, metal spatula. For treatments 4, 5 and 6, the wounding techniques were repeated and inoculated using only PDA. These last three treatments were then observed as controls. Plants were

examined daily to record the number of shoot deaths.

Resistance to fungal inoculation was measured in three ways: 1) rate of mortality, 2) average length of survival and 3) percent surviving. Cumulative amount of shoot death was plotted against the day of the experiment and the slope was calculated to represent the rate of mortality for each inoculation technique. Length of survival was calculated for each treatment by averaging the number of days each fungal-inoculated shoot remained alive in each block. The percent surviving was computed by counting the number of plants per block that were not killed by a fungal infection. An analysis of variance (ANOVA) and the least significant difference (LSD) were tabulated for each of these data sets.

Experiments on Two-year-old Hardwood Rooted Cuttings

Observations were initially made on 180 'Elliot' blueberry shoots. The shoots varied in length from 12.5-20 cm and had an approximate circumference of 1.3 cm. Shoots of similar size and age were selected from each plant. Out of the 118 plants utilized in this experiment, 44 had two different shoots inoculated by the same technique and nine had three shoots per plant inoculated. All other plants had only one shoot inoculated. Ten shoots were used per treatment in each replication. The plants were arranged in a randomized complete block design with three replications per treatment to

compare the effects of three inoculation techniques.

One of the following treatments was applied to each shoot per plant; 1) An approximate 0.60 cm flap of stem tissue was cut downward on shoots with a sterilized Bard-Parker No. 11, pointed scalpel blade to create an injury, 2) The stem tissue was pierced with a sterile Becton-Dickenson No. 5175 needle of a 10 ml syringe, 3) The same sized needle of a sterile 10 ml syringe was dipped into liquid nitrogen and pressed against the shoot to inflict a freeze injury to the stem tissue.

For treatments 1, 2 and 3, 1-2 pycnidia from the *Phomopsis vaccinii* subcultures were placed onto each stem injury with a sterilized, metal spatula. These inoculation points were then wrapped with Kim wipes that were folded and moistened with sterilized water. Parafilm was placed over the wraps to retain moisture and secure them to the stems. These mechanical-injuring techniques were repeated for treatments 4, 5 and 6 with only PDA placed on the wounded areas for mock or control inoculations.

Shoots were evaluated for symptoms of infection such as wilting or flagging, or turning brown or black in color. The amount of shoot death was recorded daily for each treatment. Wraps were removed weekly from a random selection of shoots to check for the formation of lesions or cankers. The wraps were then replaced and left on the inoculated areas until the

experiment was terminated.

Data were also collected on each inoculated shoot for; 1) its stage of growth (present season or older, woody growth), 2) whether it was flowering or nonflowering and 3) if it was on the same stem as other inoculated shoots or on stems sprouting separately from the crown.

Data from this experiment were analyzed three different ways. Cumulative amount of shoot death was plotted against the day of the experiment and the slope was calculated to represent the rate of mortality for each inoculation technique. An average length of survival and an average percent of survival per block were also calculated. ANOVAs were performed on each of these data sets along with an LSD for comparative analysis.

A second experiment was conducted to test whether leaves could be torn off as a means of inflicting injury for inoculation. Forty 'Elliot' plants were placed in a completely randomized design. One shoot per plant was inoculated with ten plants being used per treatment. The plants were subjected to one of four different treatments; 1) A leaf was torn from a shoot at the base of the petiole. Removal of the leaf caused a small injury to the stem tissue. 2) Another injury was produced by a Bard-Parker No. 11, pointed scalpel blade. For treatments 1 and 2, 1-2 *Phomopsis* pycnidia were placed at the sites of injury and wrapped as

previously described. The two techniques were repeated for treatments 3 and 4 with applications of PDA to be observed as controls. Plants were checked daily and the amount of shoot death per treatment was recorded to determine the total survivorship.

Screening Highbush Blueberry Cultivars For Resistance

Experimental Design

A factorial experiment was set up to compare the resistance of microshoots and hardwood cuttings of nine cultivars; 'Bluecrop', 'Bluejay', 'Blueray', 'Bluetta', 'Elliot', 'Jersey', 'Patriot', 'Rancocas' and 'Spartan'. Thirty rooted microshoots and rooted hardwood cuttings of each cultivar were arranged in a randomized complete block design with three replications per treatment.

Inoculation Procedures

Blueberry stem tissue was manually injured with a Becton-Dickenson No. 5175 sterile needle on a 10 ml syringe. One puncture wound was made on two distinct shoots of each plant. On two-year-old plants, the wound was made approximately 12.5 cm down from the tip of the shoot at an internode. On one-year-old plants, wounds were inflicted approximately 7.5 cm

from the tip of the shoot at an internode. Plant shoots varied between new (present season's growth) to older woody stems. An equal portion of all stem types was selected for inoculations in each block.

Each plant was subjected to two separate treatments; 1) Black pycnidia and associated mycelium from one of the *Phomopsis vaccinii* subcultures was placed on the wound of one shoot with a sterilized metal spatula, 2) The second wounded shoot of each plant was inoculated with only PDA as a control. Overall, 1080 shoots were fungal inoculated or smeared with PDA as controls. All inoculation sites were covered with several layers of moistened Kim wipes and secured with small pieces of parafilm wrapped around the stem. These wraps remained on the plants throughout the experiment.

Data Collected from Inoculations

Inoculated blueberry shoots were examined every other day for symptoms of necrosis or black and brown discolored stems. A shoot death was recognized when the stem and leaves were completely dry from the point of inoculation to the terminal end of the shoot. At the beginning of the experiment, the average length of shoots and the average number of shoots for each cultivar and age class were recorded.

Similar to the previous investigations, data from this

experiment were analyzed three different ways. A graph comparing the cumulative amount of shoot death and the day of the experiment was produced to calculate the rate of mortality for each cultivar and age class. The average length of survival was computed by measuring how long a shoot lived after fungal inoculation. The average percent of shoot survival per block was also calculated. All three calculations were analyzed by ANOVA and an LSD was generated.

Fungal Reisolation Procedures

To demonstrate that *Phomopsis vaccinii* was present in the dead, fungal-inoculated blueberry shoots, reisolations of the fungus were performed. In total, 140 stems were sampled from the various cultivars and age classes. Inoculated stems that displayed symptoms of infection were cut from the plant below the point of inoculation with sterilized scissors. Three control stems that appeared to have symptoms of fungal infection were also sampled. The stem piece was placed in a plastic bag marked with its cultivar name, age class, plant number and block. Collections of stem pieces were made over a 4 week period.

Specimens were prepared for reisolations in a Laminar flow hood. Wraps were removed from the stem pieces and the tissue was examined for any symptoms of lesions or canker

formation. The stem pieces were dipped into a beaker filled with 90 % ethanol. Surface sterilization of each stem piece was completed in a 30 % bleach solution that contained 1-2 drops of Tween 20 wetting agent for 10 minutes. After being rinsed four times with sterilized water, they were cut on a sterilized tile into three or four, 2 cm pieces by a sterilized, pointed scalpel blade. Two to three stem sections were taken from the portion of the shoot above the inoculation and at least one piece from under the inoculation site.

Difco PDA was prepared for the reisolations. The dehydrated PDA powder was put into solution and melted by heating it for 5 minutes in an autoclave at 121°C. The solution was poured into test tubes under a Laminar flow hood to a depth of approximately 3.75 cm. The test tubes containing the PDA solution were capped and placed for sterilization in an autoclave for 18 minutes at 121°C. The PDA was cooled and allowed to solidify.

One stem section was placed on PDA in each test tube under a Laminar flow hood. The test tubes were labeled with the specimen's cultivar, age class, plant number, block and the date of collection. The tubes were placed in racks and stored under cool, white florescent lamps at a PPF of 50 micromoles/m²/sec. The fungal reisolations were set aside for at least 2 weeks and then the number of growing cultures was recorded for each plant.

The presence of *Phomopsis vaccinii* in the stem sections was verified by the morphology of the fungal spores growing in the reisolation cultures. Under a Laminar flow hood, black pycnidia from each reisolation were placed on glass slides with a drop of sterilized water. A slip cover was put on top of the pycnidia and pressed down to release the spores. Fungal spores were viewed and photographed under an Olympus (BH-2) microscope with an Olympus (C-35AD-4) camera attachment (See Figure 4).

RESULTS AND DISCUSSION

Screen Development

One-year-old Microshoots

In the preliminary experiment on rooted microshoots, the first shoot deaths were observed 7 days after fungal inoculation. Additional shoot deaths were recorded for another 18 days. After this point, no more shoot deaths were observed for 2 weeks and the experiment was terminated. Four of the ten stems that were pierced with a needle survived, along with five of the shoots that were injured with a leaf tear. No deaths were observed among control plants.

Results from the second experiment with the rooted microshoots are displayed in Table 1. The first shoot deaths were recorded 6 days after inoculation. Data were collected for an additional 19 days and then no more shoot deaths were observed. The stem flap (0.36) and stem pierce (0.33) inoculation techniques had the significantly highest rate of mortality, while the leaf tear had the lowest rate (0.11). Stems that were pierced with a needle survived significantly longer (22.1 days) than the leaf tear (15.5 days) and the stem

flap (13.1 days) techniques. The highest percentage of shoots survived the leaf tear (75 % per block), while the stem pierce and stem flap techniques had the lowest percentages of survival (21 % and 17 % respectively per block).

Two control deaths (mock inoculations) were recorded; one on a stem pierce control on day no. 8 and the other on a leaf tear control on day no. 24. The death of the needle pierce control was attributed to the stem being cut too deeply by the inoculation procedure. The stem was discovered broken in two at the point of inoculation. The exact cause of the leaf tear control shoot death was not determined.

Based on these results, the stem flap and pierce techniques appeared most promising as a resistance screen. The rate of mortality for the leaf tear technique was too low and too many plant shoots survived after inoculation with *Phomopsis*. Survival rates were lower in the stem flap and stem pierce techniques. These techniques produced similar results except that the inoculated shoots survived an average of 9.0 days longer when pierced with a needle rather than when they were cut with a blade.

Two-year-old Hardwood Cuttings

The initial comparisons of inoculation techniques on rooted hardwood cuttings of 'Elliot' are summarized in Table

2. The first shoot deaths were recorded on day no. 5. Like the previous experiments, data were collected for the first 25 days. After this point no more shoot deaths were observed.

Similar to the results for the rooted microshoots, the flap technique had a significantly higher rate of mortality (0.39), a significantly lower length of survival (12.3) and a significantly lower survival percentage (20 % per block) than the other treatments. The stem freeze technique had an intermediate value for the rate of mortality (0.14) and percentage of survival (70 %). The stem pierce technique produced a low rate of mortality (0.08) and a high survival percentage (83 %), but showed an intermediate value for the length of survival (21.0 days).

One flap control death was recorded on day no. 25 of the experiment. This shoot was found snapped in two at the point of inoculation. It was surmised that the stem had been sliced too deeply by the scalpel and eventually broke in half. At the beginning of the experiment when wounds were inflicted on the stems by a scalpel, it was very easy to cut the shoots too deeply or completely in half with the blade. On occasion, alternate plants had to be chosen for the experiment because a shoot was severely damaged.

These observations suggest that the stem flap technique is not an efficient method of inoculation. The intermediacy of the stem freeze technique indicated that it should be the

treatment of choice for a disease-resistance screen, except that the liquid nitrogen was very cumbersome to handle. Since it was much easier to pierce the stem with a needle, this technique was used in further screens of blueberry varieties.

It appeared that the age of shoots and development stage did not significantly change the rate of death. The average length of survival for shoots of the present year's growth was 10.0 days; for older, woody growth, it was 9.9 days and for flowering stems, 11.6 days. It also appeared that a shoot death in one part of the plant did not contaminate another shoot or inoculation point at least for a month (the length of the experiment).

In the last study comparing scalpel flap and leaf tear wounds, the first recorded shoot death was on day No. 5 and data were collected for 25 days. Out of 10 fungal-inoculated shoots, the flap technique killed every shoot and seven shoots survived with the leaf tear technique. These results were similar to what was observed with the rooted microshoots. The flap technique killed too many shoots to be an effective screen, while too many shoots (over half) survived the leaf tear technique.

Screen of Highbush Cultivars

A summary of data from the comparison of two blueberry age classes and nine different cultivars is listed in Tables 3 and 4. The first shoot deaths were not recorded until 10 days after the fungal inoculations. For the first 4 days of the experiment the daily temperatures were approximately 16°C (60°F). These temperatures were probably low enough to slow infection of the fungus. This may explain why the first shoot deaths were recorded later than in the previous experiments where shoot deaths were first observed on days No. 5-7. For the next 7 days of the experiment, daily temperatures were between 21-24°C (70-75°F) and rates of death accelerated. This temperature range is ideal for the spread of *Phomopsis vaccinii* (Wilcox, 1939; Weingartner and Klos, 1975; Parker and Ramsdell, 1977).

Three control deaths were recorded during this experiment: one hardwood 'Bluejay' in block I, one microshoot 'Bluetta' in block I and one hardwood 'Rancocas' in block II. These shoots were examined by fungal reisolations (See below), but unfortunately stem pieces of 'Bluejay' were contaminated during the culturing process, so a possible infection with *Phomopsis vaccinii* could not be confirmed. The culture of the microshoot 'Bluetta' showed no fungal growth. *Phomopsis*

vaccinii was reisolated from the hardwood 'Rancocas' stem pieces. Since the fungal-inoculated shoot did not die on this plant and the stem marked as the control revealed a *Phomopsis* infection, it is believed that the shoot treatments were mistakenly switched. Fungal reisolations showed that one recorded control shoot death was due to an error in inoculation procedures.

Significant differences were found among cultivars in rate of mortality, length of survival and survival percentage (Table 3). 'Elliot' displayed the lowest rate of mortality (0.26) and it survived an average of 7.4 days longer than the least resistant 'Bluejay' (Table 4). 'Elliot' also had the highest survival percentage (40 %). 'Bluetta' demonstrated the second highest overall degree of resistance to *Phomopsis* canker. It averaged a 0.32 rate of mortality, 20.9 day length of survival and 27 % survival.

'Spartan' and 'Bluejay' appeared to be the least resistant. 'Bluejay' displayed the highest rate of mortality (0.44), the lowest length of survival (15.0 days) and the lowest survival percentage of only 5 %. 'Spartan' had the second highest rate of mortality (0.41) and the second lowest length of survival (16.5 days) and survival percentage (8 %).

Researchers had previously reported that 'Rancocas' has a high degree of resistance against stem canker diseases

(Childers, 1949; Weingartner and Klos, 1975). However, our data show that 'Rancocas' falls in the middle of blueberry cultivars. Its average rate of mortality equaled 0.39, average length of survival equaled 18.3 days and average survivorship totaled 17 % per block.

Figures 1, 2 and 3 are graphs of the rate of mortality for hardwood 'Elliot', 'Blueray' and 'Spartan', which appear to display the range of susceptibility for the tested cultivars. Figure 1 shows that the 30 shoots from the rooted hardwood cuttings of 'Elliot' cultivar had the lowest rate of mortality. An intermediate rate of mortality (Figure 2) was displayed by hardwood 'Blueray'. The highest rate of mortality was demonstrated by the hardwood 'Spartan' (Figure 3).

There was a significant difference between the age classes in length of survival (Table 3). The average length of survival for the hardwoods was 17.1 days and the microshoots equaled 18.9 days. There was also a significant interaction between age x cultivar for length of survival. The rankings of the most resistant ('Elliot' and 'Bluetta') and the least resistant ('Spartan' and 'Bluejay') cultivars were consistent across age classes, but the ones ranked in the middle shifted (see appendices).

Fungal reisolations from each cultivar in each block confirmed that *Phomopsis vaccinii* was present in each dead,

fungal-inoculated shoot sampled. *Phomopsis* was recovered from at least one shoot of each cultivar and age class in every block. White mycelial growth was first observed in the reisolation tubes 3 days after the cultures were started. The mycelium soon covered the surface of the PDA and grew into a thick, hairy growth in 1 week. Small, round black pycnidia appeared within 14 days. This fungal growth was characteristic of *Phomopsis vaccinii* and appeared to be the same as previous *Phomopsis* subcultures. Dr. Donald Ramsdell confirmed that the reisolations were *Phomopsis vaccinii* by examining slides made from the photographs of fungal spores from the reisolations (See Figure 4).

No cankers or lesions were discovered on the inoculated shoots when wraps were removed for surface sterilization. This could be explained by the fact that the stems were collected 30 to 50 days after inoculations. This time period was probably not long enough for the formation of lesions or cankers. Brown xylem was observed in the majority of collected stem pieces. Previous research described brown xylem as a symptom of *Phomopsis* infection (Weingartner and Klos, 1975).

SUMMARY

These experiments demonstrated that both one-year-old microshoots and two-year-old hardwood cuttings can be used for resistance screening. Stem piercing proved to be the most effective and efficient inoculation technique. All of the inoculation techniques were too labor intensive and time consuming to be utilized for a regular screen of a high number of samples. The mechanical wounding procedures have to be precisely performed or plants can be excessively damaged. Then, wraps have to be carefully placed on each inoculation point. 'Elliot' and 'Bluetta' demonstrated high levels of resistance to Phomopsis canker in our greenhouse comparisons. Further evaluation of disease resistance is recommended under field conditions, but it appears that these two cultivars could be used to improve highbush blueberry disease resistance to Phomopsis canker.

Table 1. Effect of inoculation technique on rate of mortality and survival of 'Rancocas' microshoots.

Inoculation Technique	Rate of Mortality	Length of Survival (days)	Percent Surviving
Stem Flap	0.36	13.1	17 %
Stem Pierce	0.33	22.1	21 %
Leaf Tear	0.11	15.5	75 %
LSD at 0.05 =	0.16	4.1	37 %

Table 2. Effect of inoculation technique on rate of mortality and survival 'Elliot' hardwood cuttings.

Inoculation Technique	Rate of Mortality	Length of Survival (days)	Percent Surviving
Stem Flap	0.39	12.3	20 %
Stem Freeze	0.14	23.2	70 %
Stem Pierce	0.08	21.0	83 %
LSD at 0.05 =	0.06	2.9	12 %

Table 3. F-value significance for cultivar, age class and age x cultivar after inoculation with *Phomopsis vaccinii*.

Comparison	dF	F-Value	Probability
Cultivar:			
Rate of Mortality	8	6.50	0
Average Length of Survival	8	11.21	0
Average Percent Surviving	8	4.74	0
Age Class:			
Rate of Mortality	1	2.39	0.13
Average Length of Survival	1	14.55	0
Average Percent Surviving	1	2.20	0.15
Age x Cultivar:			
Rate of Mortality	8	1.67	0.14
Average Length of Survival	8	3.70	0
Average Percent Surviving	8	1.18	0.34

Table 4. Rate of mortality, length of survival and survival percentage of blueberry cultivars after inoculation with *Phomopsis vaccinii*.

Cultivar	Rate of Mortality	Average Length Survival (days)	Percent Surviving
Elliot	0.26	22.4	40 %
Bluetta	0.32	20.9	27 %
Patriot	0.37	18.3	17 %
Blueray	0.38	18.2	13 %
Rancocas	0.39	18.3	17 %
Jersey	0.39	15.8	13 %
Bluecrop	0.40	16.5	12 %
Spartan	0.41	16.5	8 %
Bluejay	0.44	15.0	5 %
LSD at 0.05 =	0.09	2.9	20 %

Figure 1. Rate of mortality of 'Elliot' hardwood cuttings after inoculation with *Phomopsis vaccinii*.

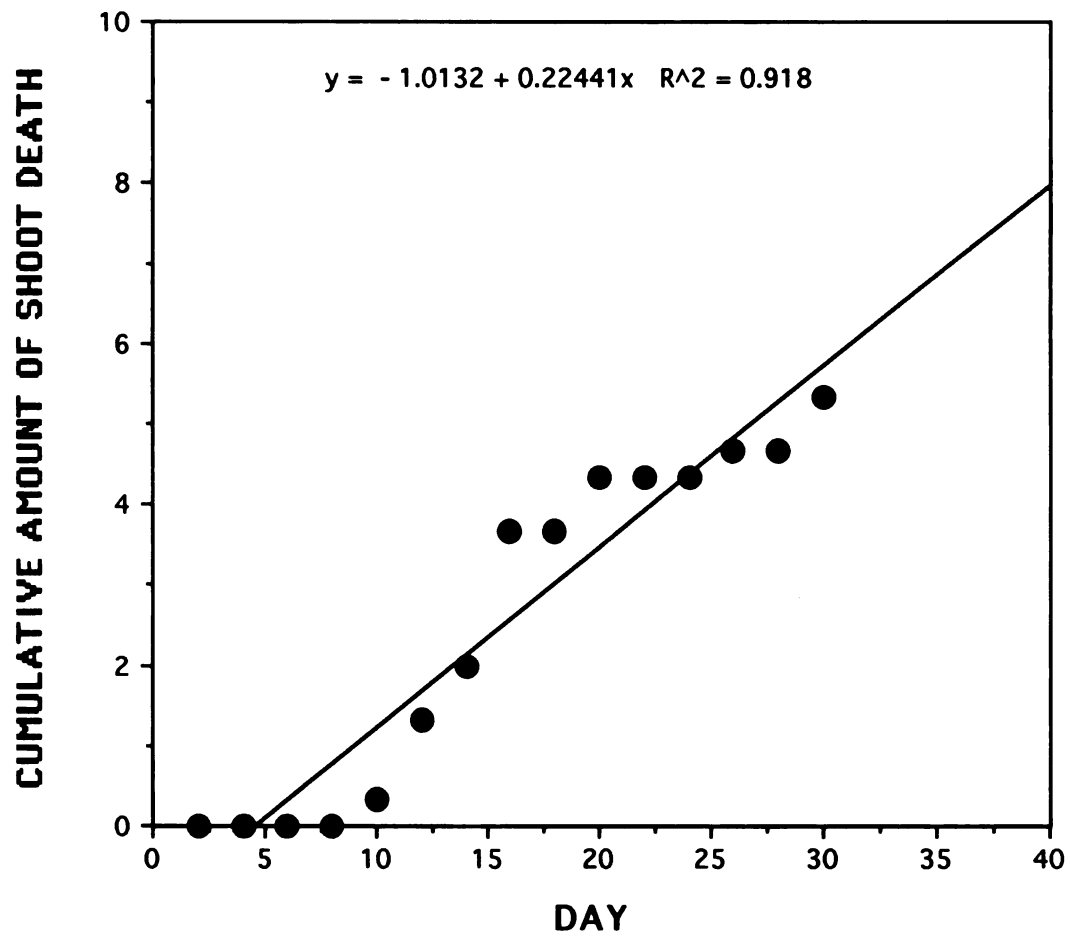


Figure 2. Rate of mortality of 'Blueray' hardwood cuttings after inoculation with *Phomopsis vaccinii*.

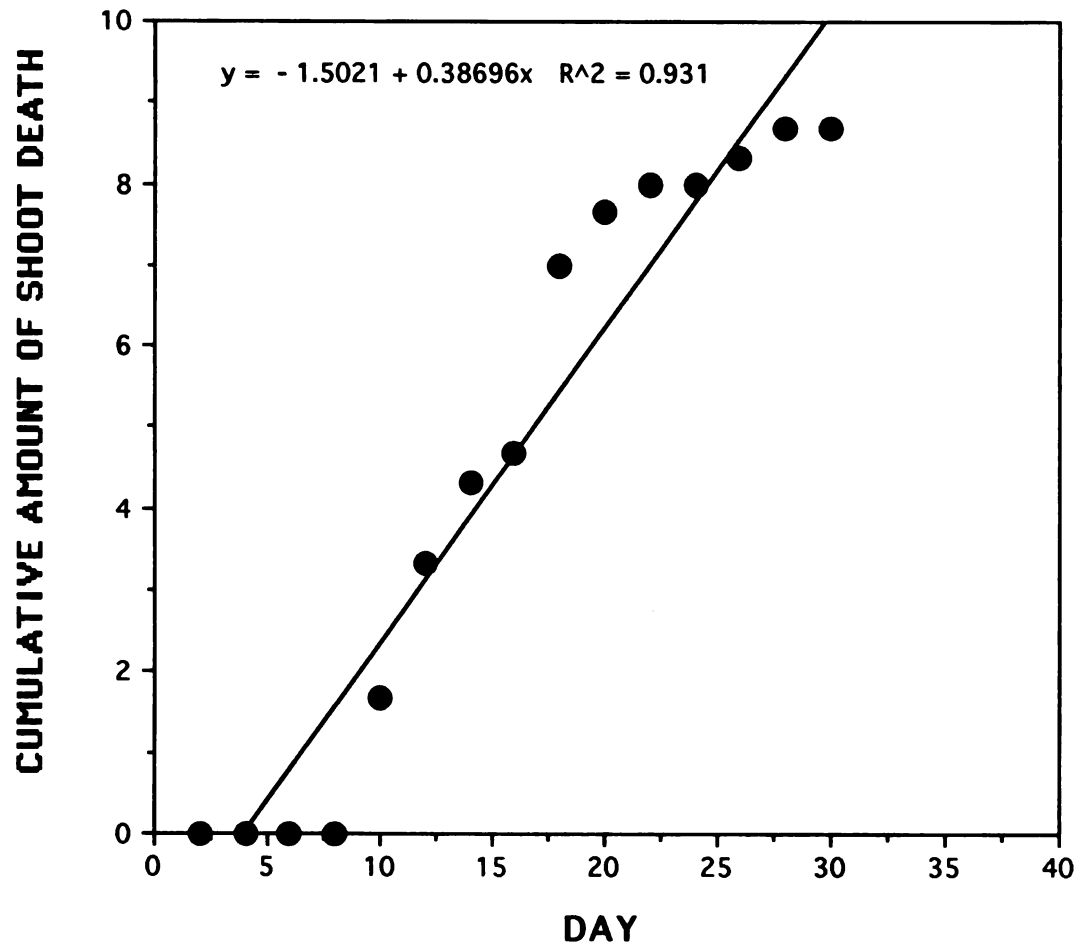


Figure 3. Rate of mortality of 'Spartan' hardwood cuttings after inoculation with *Phomopsis vaccinii*.

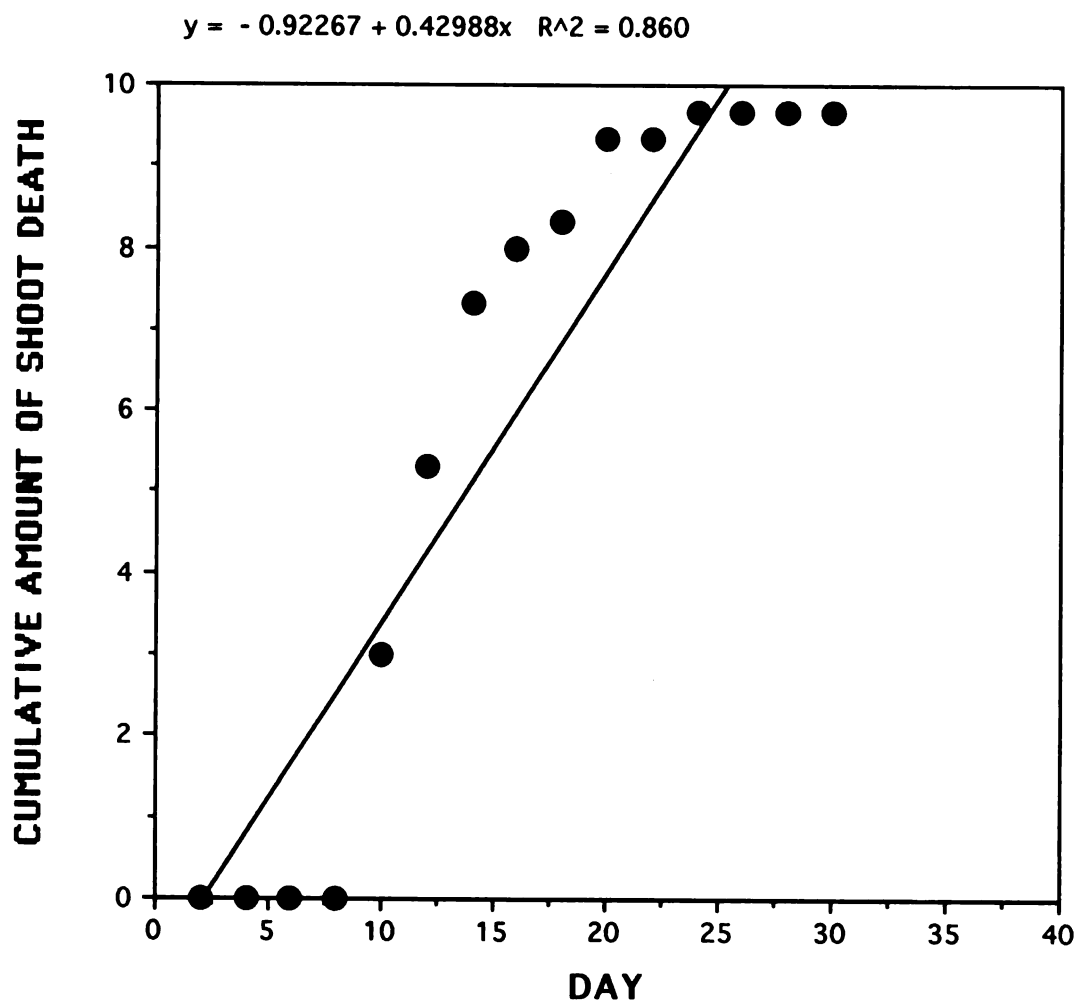
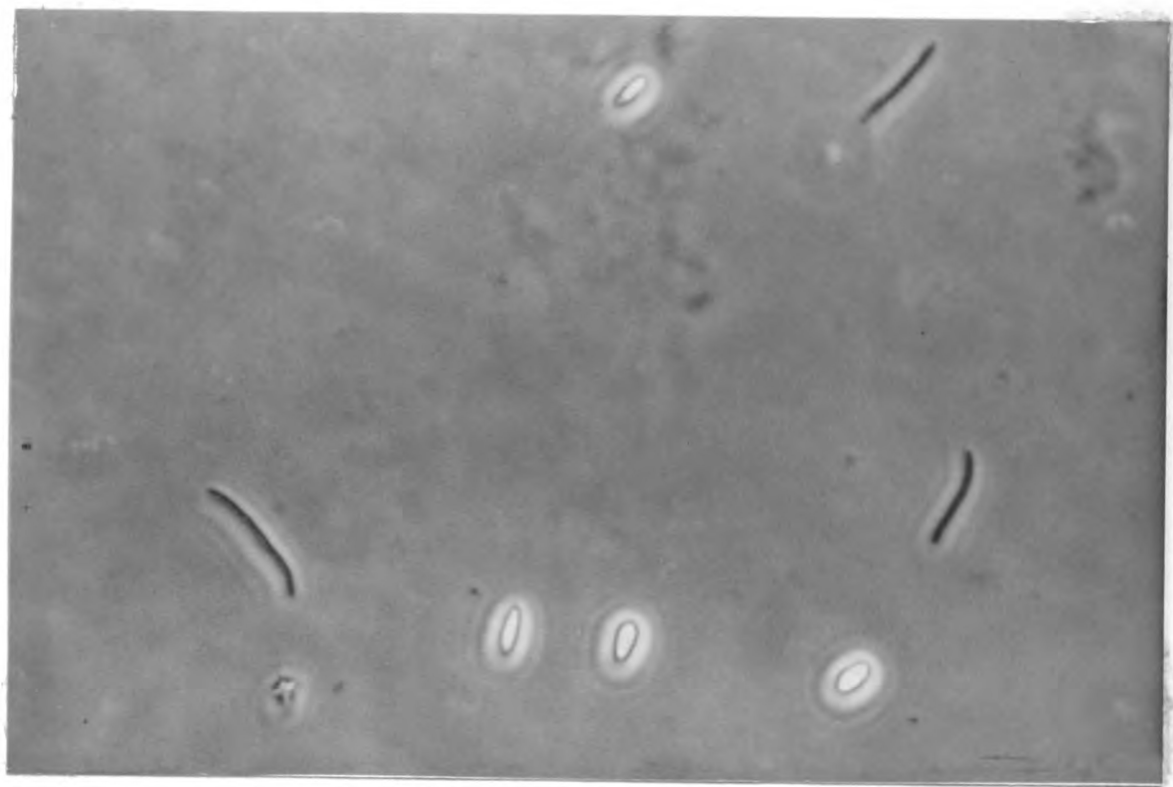


Figure 4. Photograph of alpha and beta spores
from a *Phomopsis vaccinii* reisolation.



APPENDICES

Table 5. Rate of mortality, length of survival and survival percentage of inoculated rooted microshoots of nine blueberry cultivars

Cultivar	Rate of Mortality	Length of Survival (days)	Percent Surviving
Elliot	0.28	21.5	33 %
Patriot	0.31	20.7	27 %
Rancocas	0.33	20.7	23 %
Bluetta	0.33	20.1	23 %
Jersey	0.35	16.3	20 %
Blueray	0.36	17.9	13 %
Spartan	0.37	19.0	13 %
Bluecrop	0.38	18.2	13 %
Bluejay	0.41	15.7	6 %
LSD at 0.05 =	*	1.8	*

* = F-value is not significant at $P \leq 0.05$

Table 6. Rate of mortality, length of survival and survival percentage of inoculated rooted hardwood cuttings of nine blueberry cultivars

Cultivar	Rate of Mortality	Length of Survival (days)	Percent Surviving
Elliot	0.21	23.3	47 %
Bluetta	0.28	21.7	30 %
Blueray	0.37	18.5	13 %
Bluecrop	0.39	14.9	10 %
Patriot	0.39	15.8	6 %
Jersey	0.40	15.3	6 %
Rancocas	0.40	15.9	10 %
Bluejay	0.42	14.2	3 %
Spartan	0.42	14.0	3 %
LSD at 0.05 =	0.07	1.5	15 %

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