ABSTRACT

MIRANDA, JULIE GUERRA. Epidemiological Factors Affecting Bitter Rot Infection in *Vitis vinifera* L. in North Carolina (Under the direction of Dr. Turner Bond Sutton).

Bitter rot, caused by the fungus *Greeneria uvicola* (Berk. & Curtis) Punith., is one of the most important fruit rot diseases that threaten the burgeoning winegrape (*Vitis vinifera* L.) industry in the southeastern United States. Epidemiological studies were conducted to examine the variation in aggressiveness among isolates, period of fruit susceptibility in *V. vinifera*, relative susceptibility of cultivars to bitter rot, and influence of temperature and duration of wetness on infection. Detached *V. vinifera* 'Chardonnay' fruit were inoculated with 10 isolates of *G. uvicola* obtained from fruit of *V. vinifera*, *V. rotundifolia* (muscadine grape), and a French-American hybrid. Isolates HCMD1 and HCMD5, obtained from *V. vinifera* grapes from a Maryland vineyard, were the most aggressive. Severity of disease on fruit inoculated with isolates collected from *V. vinifera* was significantly higher than with isolates collected from *V. rotundifolia*. The period of fruit susceptibility was distinguished by inoculating intact clusters of grapes in vineyards in Alamance Co. and Rockingham Co., NC, every 2 weeks from bloom until 2 weeks before harvest. Susceptibility of *V. vinifera* 'Merlot,' 'Chardonnay,' and 'Cabernet Franc' fruit increased from bloom until véraison in 2003 and from bloom until 2 weeks before véraison in 2004. The relative susceptibility of 38 cultivars and selections, including 23 *V. vinifera* cultivars and 5 French-American hybrids, was determined by inoculating and incubating detached fruit at 26°C. Fruit of *V. vinifera* were significantly more susceptible to infection by *G. uvicola* than French-American hybrids. *V. vinifera* ‘Petite Sirah,’ ‘JB97-8-0-7,’ ‘MissBlanc,’ ‘Roussanne,’ ‘Mourvédre,’ and ‘Petit Verdot’ were among the most susceptible to the bitter rot pathogen. *V. aestivalis* ‘Cynthiana Norton,’ *V. vinifera* ‘Arkansas 1271’ and ‘Riesling,’ and French-American hybrid ‘Traminette’ and ‘Chardonel’ were among the most resistant. Growth chamber studies also were conducted to examine the influence of temperature and duration of wetness on infection. Detached fruit of *V. vinifera* ‘Cabernet Sauvignon,’ ‘Cabernet Franc,’ and ‘Chardonnay’ were inoculated and incubated at 14, 18, 22, 26, or 30°C for 6, 12, 18, or 24 hours. The optimal conditions for infection of fruit by *G. uvicola* were a temperature of 23.7°C and 9 hours of wetness.

EPIDEMIOLOGICAL FACTORS AFFECTING BITTER ROT INFECTION IN *VITIS VINIFERA* L. IN NORTH CAROLINA

By

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DEDICATION

To my family, especially my parents Ricardo and Anastacia Miranda, for their love and continuing support, instilling in me the importance of an education, and inspiring me with what they themselves have been able to achieve. I love you todong todo walang break walang preno.

BIOGRAPHY

Julie Guerra Miranda was born on September 19, 1979, in Indianapolis, Indiana. While attending Cardinal Ritter High School in Indianapolis, she first became interested in a career in scientific research after participating in two National Science Foundation Young Scholars Programs. As a direct result from one of these experiences, Julie was encouraged to pursue undergraduate study with the Department of Entomology at Purdue University in West Lafayette, Indiana. During the academic year, Julie worked as a laboratory technician in Entomology while in the summers she had opportunities to intern with Dow AgroSciences LLC, American Cyanamid Company, and Bayer CropScience. She obtained her B.S. degree in Crop Protection from Purdue in 2001. She began work on a Master of Science degree in Plant Pathology at North Carolina State University under the direction of Dr. Turner B. Sutton in 2002.

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LITERATURE REVIEW

In recent years, the wine industry has expanded significantly in North Carolina and other states in the southeastern United States. New growers, planting in areas without viticultural traditions, are faced with a wide range of unusual problems relating to the climate and the large reservoir of endemic pathogens and pests flourishing on the abundant population of wild vines (McGrew, 1982). Higher costs and greater risks meet these growers who must contend with costs of pesticides and their application, losses due to pests and disease despite sprays, and the usual labor and operating expenses in addition to the significant investment represented in vineyard establishment. With the warmer climate, high humidity, and in particular, the potential for frequent rainfall when fruit are nearing harvest, bitter rot is one of the diseases winegrowers must be prepared to manage.

The bitter rot problem challenges both growers and researchers alike in that literature on the causal organism, *Greeneria uvicola*, is lacking and outdated. Early research focused only on identification and control. The few studies in epidemiology have been conducted primarily on grape species other than *Vitis vinifera*, which is of most importance to the wine industry and cultivated around the world.

1.1 Distribution, importance, and host range of *Greeneria uvicola.* Bitter rot of grape, is caused by the fungus *Greeneria uvicola* (Berkeley & M.A. Curtis) Punithalingam (syn. *Melanconium fuligineum* (Scribner & Viala) Cav.) (Sutton & Gibson, 1977). The disease is common to the eastern United States, south of

Pennsylvania and west to Texas, and has occasionally been found as far north as the Finger Lakes region of New York (Pearson & Goheen, 1988). Although bitter rot is not a problem in France or Germany, the disease has been recognized in Italy and Greece (McGrew, 1977). Bitter rot has also caused losses in the grape-growing regions in Japan, Taiwan, China, India, Australia, New Zealand, Costa Rica, Brazil, and South Africa (Abrahão et al., 1993; Ridings & Clayton, 1970; Sutton & Gibson, 1977; Wu & Chang, 1993; Yan et al., 1998).

Yield reduction due to bitter rot has been reported as much as 30% in North Carolina (Clayton, 1975; Ridings & Clayton, 1970) and as high as 50% in severely affected areas of the world (Abrahão et al., 1993). Besides reduced yield, losses are compounded because wine, produced from fruit with as little as 10% infection, can be bitter and undrinkable (hence the name bitter rot) (McGrew, 1977). Infections on fruit can reduce the market value of grapes intended for fresh consumption by causing blemishes and additional deterioration during transport and storage (Reddy & Reddy, 1983). In grape-growing regions of Greece and India, infections of *G. uvicola* on fruit and canes can cause girdling and dieback of young shoots (Critopoulos, 1960; Prakash et al., 1974).

Bitter rot attacks many species of grape, including *Vitis vinifera* L. (European wine grape), *V. labrusca* L. (fox grape), and *V. aestivalis* Michx. in the subgenus *Euvitis* as well as *V. rotundifolia* Michx. (muscadine grape) in the subgenus *Muscadinia* (Sutton & Gibson, 1977; Farr et al., 2001). Ridings & Clayton (1970) indicated that pathogenicity of *G. uvicola* was not restricted to grape, and the fungus was capable of infecting mature fruit of apple, cherry, strawberry, peach, and banana as well as immature blueberry fruit when inoculated under laboratory conditions. The pathogen also attacks rambutan postharvest in Thailand (Farungsang et al., 1998).

1.2 Taxonomy and nomenclature. Although attempts at finding a sexual stage of the fungus have failed (Ridings & Clayton, 1970), molecular sequence data determined *G. uvicola* belongs in the order Diaporthales within the class Pyrenomycetes within the phylum Ascomycota (Farr et al., 2001). Members of the Diaporthales, which include ~ 98 genera of plant-associated fungi, have asexual states that are generally coelomycetous bearing phialidic conidiogenous cells and conidia in acervuli or pycnidia with or without a well-developed stroma (Farr et al., 2001). Acervuli of *G. uvicola* on fruit are 200 to 550 µm in diameter, light brown, subepidermal, separate, and often develop in concentric circles (Farr et al., 2001; Reddy & Reddy, 1983; Sutton & Gibson, 1977). Conidiophores are branched, produced in a compact layer, hyaline, and taper towards the apices (Farr et al., 2001; Kao et a., 1990; Reddy & Reddy, 1983; Sutton & Gibson, 1977; Ullasa & Rawal, 1986). Conidia of *G. uvicola* are pale brown, smooth, thin-walled, aseptate, fusiform to ovoid, base truncate, apex obtuse, and ~ 6 to 13 µm x 2 to 5µm (Farr et al., 2001; Kao et al., 1990; Sutton & Gibson, 1977; Ullasa & Rawal, 1986). Colonies on potato dextrose agar (PDA) are characterized by a dense mycelium with a cottony surface, gray in color, adpressed with scanty aerial growth, and develop numerous dark green to black masses of conidia scattered on the surface (Farr et al., 2001; Reddy & Reddy, 1983).

Greeneria is a monotypic genus based on *G. fuligineum*, which was described by F.L. Scribner and P. Viala (1887). F. Cavara (1888) later transferred the species to *Melanconium*, and for years, the fungus appeared as *M. fuligineum* in the literature. E. Punithalingam (1974) determined that M.J. Berkeley and M.A. Curtis provided an earlier epithet in *Phoma uvicola*, recognized it was dissimilar from other *Melanconium* species, transferred the earlier epithet to *Greeneria*, and proposed the species *G. uvicola*.

1.3 Winegrape production in the United States and North Carolina.

Grapevines grow abundantly in the United States, and a greater selection of cultivars can be grown here than in any other country. Grapes are grown for commercial wine production in 44 states (Vine, 1997). Explorers made note of wild grapevines in 1524 in what is now North Carolina (Vine, 1997), and a record of wine production from *V. rotundifolia* exists as far back as 1565 (Milholland, 1991). Today the United States ranks 4th in worldwide production of wine with 75% of wine consumed by Americans produced domestically (Vine, 1997). Although California dominates the American industry, the number of small winemakers and winegrowers continues to increase in the eastern United States (Vine, 1997).

The North Carolina Grape Council, created in 1986, was given the responsibility of stimulating the growth of the state's industry by sponsoring research, education, and promotion (Wolf & Poling, 1996). According to the North Carolina Department of Agriculture & Consumer Services (NCDA&CS, website: http://www.ncwine.org), the state ranked 12th in the United States in wine production as well as grape production and acreage in 2003. Approximately 2500 metric tons of grapes and 2 million liters of wine, estimated at a value of $30 million, were produced in North Carolina in 2003. The rapid growth of the industry has been evidenced by the state's 300 vineyards in 2003, up dramatically from 200 just two years previously. The state's 31 wineries are expected to expand by another 10 before September 2005. Much of the development is targeted toward tourism, and winegrowing is being considered for farm diversification and as a possible alternative crop to tobacco. North Carolina recently gained added recognition in 2003 when the federal government designated the Yadkin Valley as the state's first American Viticultural Area (AVA), which identifies and allows the seven-county section to market itself as a distinct grape-growing region (NCDA&CS, 2004).

Although sweet wine has traditionally been produced from *V. rotundifolia* in North Carolina, the recent growth has been primarily focused on producing European-style dry wines. The most popular cultivars in the expansion have included *V. vinifera* 'Chardonnay,' 'Cabernet Sauvignon,' 'Merlot,' 'Viognier,' and 'Cabernet Franc' (NCDA&CS, 2004). Many vineyard plantings have been trained to bilateral cordon trellising with vertical shoot positioning, but recent recommendations have started to encourage lyre trellising or other divided canopy systems in order to support the vigorous growth of the vines in North Carolina's climate.

Pierce's disease, caused by *Xylella fastidiosa*, is the principal limiting factor in the production of *V. vinifera* in the southeastern United States (Wolf & Poling, 1996; Southeastern Grape IPM, http://www.cals.ncsu.edu/plantpath/ExtensionPro/grapes/, 2004). Consequently, the majority of *V. vinifera* plantings in North Carolina have been restricted to the cooler foothills of the Appalachian Mountains. Other important diseases include downy mildew, powdery mildew, and crown gall. The humid climate of the region also makes fruit susceptible to a host of rots, including Botrytis bunch rot, black rot, Phomopsis, ripe rot, sour rot, and of course, bitter rot (Wolf & Poling, 1996).

1.4 Disease cycle of *G. uvicola* on grapes. The fungus *G. uvicola* overwinters on canes, mummies, and other plant debris (Critopoulos, 1960; Kummuang et al., 1996a). In the spring, black masses of conidia exude from acervuli. Conidia formation on *V. vinifera* vines was found to be abundant from April to June in Japan (Kato et al., 1978). Spores are splash-dispersed by rain and other sources of water and can cause infection on all green parts of the plant (Luttrell, 1953). In *V. rotundifolia* vineyards*,* the highest peak of conidia release occurred 1 week prior to the time that bitter rot symptoms were prevalent on leaves and coincided with the highest peak of the disease on fruit (Kummuang et al, 1996a). Flecking symptoms may develop on lush vegetative tissue including tendrils, young shoots, flower buds, and leaves when emerging from buds. Leaves are penetrated directly, not through stomatal openings, and flecking can form with equal intensity on both the abaxial and adaxial surfaces of the leaf (Ridings & Clayton, 1970). Severe flecking can progress into a blight of small berries, though acervuli were not observed in the flecks, and can result in berry drop (Kummuang et al., 1996b). However, flecking on *V. vinifera* is considered rare and is not thought to contribute significantly to disease development (Luttrell, 1953).

More importantly, the spores are splashed onto the pedicels, where the fungus invades but remains latent until the fruit begin to ripen (Kummuang et al., 1996b). *G. uvicola* could be isolated from the *V. rotundifolia* fruit throughout the season, leading researchers to conclude colonization without symptom expression is prevalent, especially during the later part of the season (Kummuang et al., 1996b). Olive or brown lesions develop into a soft rot of the ripening fruit. Fruit of *V. labrusca* inoculated at pea stage, berry touch, after berry touch, and beginning of véraison in Taiwan did not develop symptoms until after 12°Brix, a measurement of soluble solids (Kao et al., 1990). Bitter rot symptoms were also produced in the laboratory on half- and full-sized fruit of *V. rotundifolia* (Kummuang et al., 1996b).

Secondary infections can occur where fruit has been damaged by bird feeding or by splitting or cracking of berries due to rain (Pearson & Goheen, 1988). Insect injury has also been implicated in the creation of an infection site (Reddy & Reddy, 1983). Ridings & Clayton (1970) found insect wounds associated with approximately 30% of *V. labrusca* fruit that had natural infections of bitter rot. The highest percent of infection was obtained in the laboratory when *V. rotundifolia* fruit were wounded and inoculated with a mycelial plug (Kummuang et al., 1996b). In the field, bitter rot did not develop on muscadine fruit inoculated several times during the season except in wounded, immature fruit with pH 2.7 and ~ 5°Brix (Ridings & Clayton, 1970). Mycelium also spreads within the cluster but often leaves a few berries unaffected; the fungus can also spread berry-to-berry through peduncle branches as well as by direct contact between healthy and diseased fruit if moisture is sufficient for spore germination (Critopoulos, 1960). The fungus can directly penetrate the epidermis of the fruit via formation of a hyaline appressorium and penetration peg (Kao et al., 1990), and hyphae colonize the fruit tissue intracellularly (Kummuang et al., 1996b).

Rotting fruit become covered in characteristic concentric rings of acervuli. Sporulation (Critopoulos, 1960) and mycelial growth (Ridings & Clayton, 1970) occur more rapidly in mature fruit. The ability of *G. uvicola* to cause rot may result from the production of enzymes since the fungus has been shown to produce polygalacturonase and transeliminase in culture in both glucose-sodium polypectate (GSP) and pectin media (Ridings & Clayton, 1970). The infected fruit eventually shrivel into mummies. Clinging to the vine or fallen to the ground, mummies and infected fruit spurs and pedicels sporulate the next season with conidia that serve as primary inoculum (Kummuang et al., 1996a).

1.5 Symptoms and signs. The fungus infects all above-ground vegetative parts of the grape plant. Leaf infections occur as tiny, sunken reddish-brown flecks with yellow haloes. Lesions on stems and petioles are round to elliptical, slightly raised, and reddish-brown to black in color (Luttrell, 1953). Flecking of the sepals and blighting of the flower buds can also occur. Russetting of young and developing fruit has been associated with early *G. uvicola* colonization of *V. rotundifolia*. A netlike or starfish russet pattern develops initially around the pedicel and spreads further down the muscadine fruit until eventually the russet progresses into typical bitter rot symptoms (Kummuang et al., 1996b). Infected berries soften and become completely covered with concentric rings of acervuli (Fig. 1), and the underlying tissue becomes necrotic (Reddy & Reddy, 1983).

Light-colored fruit may become brown while dark-colored fruit take on a roughened, sparkly appearance when black acervuli begin to develop (Pearson & Goheen, 1988). Infected fruit may fall to the ground or remain attached as mummies. The rotted, shriveled fruit are often confused with berries affected by Phomopsis, black rot, or ripe rot (Farungsang & Farungsang, 1992b; Kao et al., 1990; McGrew, 1977; Pearson & Goheen, 1988).

1.6 Environmental conditions favoring bitter rot. Ridings & Clayton (1970) studied the growth of isolates of *G. uvicola* obtained from fruit and cultured on PDA at temperatures ranging from 2 to 40°C. The optimal range of temperatures for mycelial growth was between 28 and 30°C and for sporulation was between 8 and 24°C (Ridings & Clayton, 1970). Critopoulos (1960) found similarly that isolates collected from woody tissue grew on PDA in temperatures from 22 to 30°C. The fungus does not grow or survive at 40°C (Ridings & Clayton, 1970). No spores germinated at 8°C or 12°C within 20 hours, whereas 22 and 62% of conidia germinated at 16°C and 20°C, respectively. Complete spore germination occurred from 24 to 36°C after 20 hours, and no difference was found in the percent of germination or germ tube length between 28 and 32°C. Incubation of spore suspensions for 10 minutes at temperatures from 30 to 45°C resulted in 100% germination, but viability decreased rapidly if spores were incubated in water for more than 7 hours at 45°C (Ridings & Clayton, 1970).

When detached *V. rotundifolia* ‘Magnolia’ and ‘Topsail’ fruit were inoculated in the laboratory, bitter rot development was greatest at 28°C with 80% infection (Ridings & Clayton, 1970). Kao et al. (1990) found similar results where infection peaked at temperatures ranging from 24 to 28°C in detached fruit of *V. labrusca* 'Kyoho.'

Kummuang et al. (1996a) examined the effects of temperature and rainfall on the occurrence of bitter rot in muscadine grapes. Total rainfall in three vineyards in Mississippi ranged from 26 to 65 cm for June, July, and August in 2 years. Temperature and rainfall had a non-linear effect on incidence, and the model had a low R^2 value. Rainfall in early summer correlated with high incidence at harvest. However, rainfall was also hypothesized to not have as strong an effect as temperature due to the location of muscadines in the canopy (Kummuang et al., 1996a). Related studies confirmed the importance of moisture in disease development. Field infections during rainy seasons in muscadine fruit occurred when temperatures were between 12 and 30°C (Milholland, 1991). Infected canes in Greece died more rapidly immediately after irrigation (Critopoulos, 1960), and bitter rot endemic to a vineyard did not appear in a drought year in India (Reddy & Reddy, 1983).

Ridings & Clayton (1970) and Ullasa & Rawal (1986) found that germination of conidial suspensions of some *G. uvicola* isolates in water was poor. Ridings & Clayton (1970) examined the effect of additives after spores failed to germinate following 20 hours incubation in water at 30°C. Casein hydrolysate or yeast extract in aqueous solutions stimulated spores to germinate within 20 hours, but asparagine, dextrose, glycine, ammonium chloride, or thiamine were ineffective (Ridings & Clayton, 1970). Ullasa & Rawal (1986) also found increased germination using suspensions of leaf or fruit extract for ~ 22 hours.

Isolates of *G. uvicola* cultured on PDA survive successfully under acidic conditions. Spore germination occurred from pH 3.2 to 7.5 whereas mycelial growth was optimal at 30°C with pH ranging from 2.3 to 3.5 (Ridings & Clayton, 1970). These results were supported by Kao et al. (1990) who found that infections in the field increased when pH was between 3.4 and 4.0 and Kyoho fruit was >13°Brix. Inhibition of growth by acids predominant in muscadine fruit was highest with levels of 0.8 and 1.6% DL-malic + 0.4% D-tartaric amendments in media (Ridings & Clayton, 1970).

1.7 Cultivar susceptibility to bitter rot. Since the type of grape from which a wine is fermented strongly determines its final characteristics, quality wines, known as "varietals," are often highly associated with a single grape cultivar (Vine, 1997). Since variety is so important to the identity of a wine, growers are reluctant to change cultivars, while at the same time, there exists a disparity of information regarding the susceptibility of popular cultivars to infection by *G. uvicola*.

***1.7.1 Susceptibility of* V. rotundifolia.** Literature on relative susceptibility of grapes to the bitter rot fungus is most abundant on cultivars of *V. rotundifolia* (i.e. muscadine grape). The thick-skinned fruit develop in small, loose clusters, are usually consumed fresh, and occasionally are used for making sweet wines in the southeastern United States (Vine, 1997).

Based on 8 years of observations and counts, Clayton (1975) found *V. rotundifolia* 'Magnolia' most susceptible. *V. rotundifolia* 'Roanoke,' 'Scuppernong,' and

‘Carlos’ were moderately susceptible, and *V. rotundifolia* ‘Pamlico,’ ‘Chowan,’ ‘Higgins,’ ‘Hunt’, and ‘Albemarle’ were slightly resistant. Additionally, laboratory inoculations showed that *V. rotundifolia* ‘Thomas,’ ‘Topsail,’ and ‘NC57-56’ fruit also were susceptible to infection by *G. uvicola* (Ridings & Clayton, 1970). Although losses on muscadines were hypothesized to be less in general than on *V. vinifera*, planting these cultivars for high-yield commercial use in North Carolina was still discouraged unless disease control practices were implemented (Clayton, 1975).

Infections by *G. uvicola* caused varied symptoms in different muscadine cultivars. Kummuang et al. (1996b) reported that leaf flecking was more severe on *V. rotundifolia* ‘Sterling’ and ‘Carlos’ than on ‘Doreen.’ These same cultivars differed in the degree of russeting of the fruit, which the researchers hypothesized was a resistance expression of the fruit to bitter rot development. Sterling was most susceptible to infection by *G. uvicola* on leaves and on fruit. In the field, bitter rot incidence was higher on fruit of Carlos, Sterling, and Cowart, and berries dropped earlier on Sterling and Cowart than on Doreen (Kummuang et al., 1996b).

1.7.2 *Susceptibility of* V. vinifera. The high quality of fruit of *V. vinifera* (European wine grape) makes it attractive for cultivation around the world primarily for wine production but also as a table grape (Pearson & Goheen, 1988; Vine, 1997). The species is extremely susceptible to all American pests and diseases (Pearson & Goheen, 1988), but the few studies that have examined susceptibility of *V. vinifera* to infection by the bitter rot fungus have done so using cultivars popular in countries other than the United States.

In Greece, *V. vinifera* 'Savatiano,' 'Roditis,' 'Fileri,' and 'Monemvassia' were most susceptible to girdling of the canes by *G. uvicola* (Critopoulos, 1960). *V. vinifera* 'Razaki' also was susceptible though symptom development occurred more slowly, and *V. vinifera* 'Sideritis' and 'Fraoula' were the least susceptible of the cultivars tested. Bitter rot infection also occurred when the fruit of Sideritis, Razaki, Savatiano, Fileri, and *V. vinifera* 'Siriki' were inoculated with an aqueous suspension of spores on an unwounded surface (Critopoulos, 1960). However, other reports suggest wounding (Reddy & Reddy, 1983) or the addition of a germination stimulant (Ridings & Clayton, 1970) may be necessary for bitter rot infection to occur on other *V. vinifera* cultivars.

Natural bitter rot infections have been observed in *V. vinifera* 'Anab-e-Shahi,' 'Black Champa' (Reddy & Reddy, 1983; Ullasa & Rawal, 1986), 'Angur Kalan,' 'Kali Sabehi,' 'Taifi Rosovi,' 'Thompson Seedless,' 'Jaos Beli,' 'Khadari,' 'Pandri Sabehi,' and 'Gulabi' vineyards in India (Reddy & Reddy, 1983). Peduncle infections of Anab-e-Shahi resulted in berry drop, and withered leaves remained attached to vines with girdled canes in laboratory inoculations (Prakash et al., 1974). Ullasa & Rawal (1986) reported Anab-e-Shahi, Black Champa, Thompson Seedless, and *V. vinifera* 'Bangalore Blue' were so highly susceptible to berry rot and leaf and twig blight caused by *G. uvicola* that dieback symptoms of the canes resulted. No infection of *V. vinifera* 'Perlette' and 'Corna Rosea' was present under field conditions in India (Reddy & Reddy, 1983). The bitter rot fungus has also been isolated from *V. vinifera* 'Italia' in Taiwan (Kao et al., 1990) and from 'Semillon' in Australia, where *G. uvicola* was one of the fungi associated with dieback in New South Wales (Castillo-Pando et al., 2001).

1.7.3 ***Susceptibility of* V. labrusca *and other cultivars.*** Although of primary importance as a North American table and juice grape, *V. labrusca*, or the fox grape, is also occasionally used for production of wine (Vine, 1997). In the field, bitter rot has been found naturally developing on *V. labrusca* 'Catawba' (Bordelon & Moore, 1982; Moore & Bordelon, 1982; Moore et al., 1980; Moore & Micinski, 1980; Moore & Schroeder, 1983a, 1983b), 'Kyoho,' and 'Niagara' (Kao et al., 1990). In laboratory inoculations, *V. labrusca* 'Niagara' and 'Portland' also were susceptible to bitter rot infection (Ridings & Clayton, 1970).

The following inter-specific hybrids have been found to be susceptible to bitter rot: *V. aestivalis-cinerea* x *V. vinifera* 'Jacquez'(Abrahão et al., 1993); *V. vinifera* x *V. labrusca* 'Golden Muscat' and hybrid 'Black Queen' (Kao et al., 1990); and *V.* x *aestivalis* Warren (Luttrell, 1953). McGrew (1977) found *V. labrusca* x *V. vinifera* 'Seneca' to be resistant.

1.8 Management of bitter rot in grapes. Management of bitter rot has been most effective through a combination of methods. Studies have demonstrated chemical and cultural controls to be successful whereas few biological control options have been developed. Whether relating to the cost differences between fungicides and number of applications, or the amount of labor required, economics is often an important consideration for the disease management decisions of a wine grower.

1.8.1 ***Cultural control.*** Pruning vines during the dormant season and removing mummies and other plant debris will help reduce the amount of initial inoculum and disease severity (Kummuang et al., 1996a; Milholland, 1991; Wolf & Poling, 1996). However, in areas where the disease is severe, such as Taiwan, pruning in the spring stimulates vines into budding when spores were present, resulting in infections of the green tissue and dead arm symptoms (Kao et al., 1990).

During the growing season, Milholland (1991) suggested that removing excess shoot growth and foliage would aid pesticide application and reduce leaf wetness. Weed control and good canopy management, which includes not just pruning but also extends to leaf removal and shoot positioning in grapevines, increases air circulation and shortens the time for drying. Abrahão et al. (1993) studied the combined effects of canopy pruning and fungicides on the very vigorous *V. aestivalis-cinerea* x *V. vinifera* 'Jacquez' vines in Brazil. Canopy pruning resulted in lower incidence of bitter rot in mature fruit and diseases in general in all the treatments. The reduction in foliage also allowed for better penetration of fungicides into the fruit cluster (Abrahão et al., 1993).

Since the symptoms of bitter rot do not develop until late in the season, fruit that remains on the vine past optimal harvest will be vulnerable to bitter rot infection. Consequently, in anticipation of approaching rain or even severe weather events and having to juggle a tight harvest schedule, growers may be pressured to avoid the disease by harvesting earlier and before sugar and acidity balance is optimum. McGrew (1982) suggested this method of avoidance might explain the reputation of high acidity in wines from the eastern United States.

1.8.2 ***Biological control.*** Wu & Chang (1993) investigated the potential for biological agents to control bitter rot in Taiwan. Of the 46 microorganisms isolated from grape fruit, stem, and leaf tissues, two Gram-positive bacterial isolates, designated F3 and M5, were found to be the most effective in inhibiting *G. uvicola* growth during *in vitro* screening. Hyphae of *G. uvicola* affected by the bacterial isolates became swollen or malformed, lost cytoplasm, and formed abnormal vesicles. When applied as a bacterial suspension 24 hours prior to *G. uvicola* inoculation, M5, identified as a *Bacillus* sp., and F3 provided significant control of bitter rot on detached clusters of *V. labrusca* 'Kyoho' in the lab. Applying suspensions of M5 every 10 days also provided adequate control of bitter rot in field trials although it was not as effective as a standard treatment with mancozeb. *Bacillus* sp. M5 was hypothesized to produce an antibiotic and have competitive and even hyperparasitic abilities, resulting in control of bitter rot at pre- and postharvest stages (Wu & Chang, 1993). Biological control of bitter rot with phylloplane yeasts was also attempted in Thailand where *G. uvicola* causes a postharvest problem on rambutan (Farungsang et al., 1998). Although these tests suggest there is potential for successful biological control of bitter rot in the field, at this time commercial products are neither available nor are they known to be under development.

1.8.3 ***Chemical control.*** Ridings & Clayton (1970) determined the relative toxicity of 14 fungicides on spore germination and mycelial growth *in vitro*. Conidia of *G. uvicola* were more sensitive than mycelia to fungicides, and only the benzimidazole fungicide benomyl was more effective on mycelial growth than spore germination. Metiram, zineb, folpet, and captan, were ineffective at inhibiting mycelial growth, whereas maneb and mancozeb were both highly effective on limiting spore germination and mycelial growth (Ridings & Clayton, 1970).

Management of bitter rot in the field has been most successful when protectant fungicides are used prior to the warm, wet weather periods favorable for infection. Generally, early season bitter rot is managed concurrently with other diseases like black rot (McGrew, 1977). Bitter rot has also been observed to be more prevalent where protective sprays for downy mildew control were not applied properly (Critopoulos, 1960). However, late season and preharvest fungicides for bitter rot should be applied, especially during a rainy harvest season or if harvest is delayed past maturity (McGrew, 1982; Kao et al., 1990).

Early studies comparing chemical control options for bitter rot in *V. rotundifolia* found that spray programs including dithiocarbamates, benomyl, and captan were most effective (Ridings & Clayton, 1970; Clayton, 1975). Recent tests have shown Quinone Outside-binding Inhibitors (QoI), including azoxystrobin and trifloxystrobin, are effective in controlling bitter rot in *V. rotundifolia* 'Triumph' and 'Fry' in North Carolina (Cline & Bloodworth, 2001a & 2001b).

Fungicidal control of bitter rot in *V. vinifera* has also been investigated. Of eight fungicide and canopy defoliation combinations, benomyl was most effective in controlling rot, and thiophanate-methyl + chlorothalonil least effective in the Jacquez cultivar in Brazil (Abrahão et al., 1993). In North Carolina, Harrison et al. (2002) experienced moderate success controlling bitter rot with a treatment of copper sulfate and sulfur in organically managed Chardonnay and Cabernet Sauvignon. In the same study, QoI-based treatments, including trifloxystrobin, azoxystrobin, or kresoxim-methyl, and a captan-based standard spray program provided similar control, although fewer clusters of Cabernet Sauvignon were affected in the QoI programs than the standard (Harrison et al., 2002). The incorporation of QoI fungicides into a captan-based program also provided excellent control under high disease pressure in *V. vinifera* 'Merlot' (Sutton et al., 2004).

Dormant sprays of pentachlorophenol + lime sulfur on *V. vinifera* inhibited sporulation on vines in Japan (Kato et al., 1978). During the growing period, benomyl and especially early applications of thiophanate-methyl in May and June were most effective in controlling bitter rot. Wettable powder formulations of copper and maneb were also effective, but phytotoxicity was observed (Kato et al., 1978).

Current recommendations for wine grapes in North Carolina advise spraying for bitter rot at postbloom, or 10 to 14 days after the prebloom spray, with mancozeb + a triazole (e.g. myclobutanil or tebuconazole) or QoI fungicide (e.g. azoxystrobin, kresoxim-methyl, or trifloxystrobin) (Sutton & Sorensen, 2004). The first cover spray, 10 to 14 days after the postbloom application, should consist of captan or mancozeb + a triazole fungicide. Captan is recommended again for additional cover sprays throughout the growing season on a 10 to 14 day interval. Growers should make a final application of captan or a QoI fungicide 10 to 14 days prior to harvest while paying particular attention to preharvest intervals (Sutton & Sorensen, 2004). Since postbloom and preharvest occur at the beginning of June and August, respectively, in North Carolina, these recommendations differ from Kato et al. (1978) who found fungicidal applications more effective from May to June.

Dithiocarbamates, in particular mancozeb, and benzimidazoles, specifically benomyl, have overall provided the most consistent control of bitter rot in *V. labrusca* fruit (Bordelon & Moore, 1982; Moore & Bordelon, 1982; Moore & Micinski, 1980; Moore & Schroeder, 1983). Full season spray programs with triadimefon alone and in combination with metalaxyl reduced bitter rot infection in Catawba fruit in Missouri (Moore & Schroeder, 1983).

Ullasa & Rawal (1986) examined 10 fungicides applied as a postharvest dip on *V. vinifera* Anab-e-Shahi fruit against rot in storage. Where there was a high incidence of bitter rot in the untreated control, captan and captafol were most effective. Treatments with carbenzadim and chlorothalonil were moderately effective in controlling postharvest bitter rot (Ullasa & Rawal, 1986).

Chemicals have provided excellent control of bitter rot, but rotation of fungicides is important to guard against the development of resistant populations. Although there have been no reports of fungicide-resistant populations for bitter rot on grape, *G. uvicola* isolates collected from rambutan postharvest in Thailand were frequently found to be resistant to benomyl (Farungsang & Farungsang, 1992a; Farungsang, N., *personal communication*).

INTRODUCTION

Bitter rot of grape, caused by the fungus *Greeneria uvicola* (Berk. & Curtis) Punith. (syn. *Melanconium fuligineum* (Scrib. & Viala) Cav.), attacks many *Vitis* species, including *V. vinifera* (European wine grape), *V. rotundifolia* (muscadine grape), and *V. labrusca* (fox grape). The disease occurs in the eastern United States and the grape-growing regions of at least 10 other countries (Sutton & Gibson, 1977; Wu & Chang, 1993; Yan et al., 1998). The bitter rot fungus invades pedicels in the spring, and latent symptoms are expressed as olive or brown lesions that develop into a soft rot of the ripening fruit. Yields have been reduced by as much as 30% by bitter rot in North Carolina (Clayton, 1975; Ridings & Clayton, 1970) and up to 50% in areas of the world where the disease is severe (Abrahão et al., 1993).

In recent years, the wine industry has expanded significantly in North Carolina and in the eastern United States. Much of the expansion is by small growers and is targeted toward tourism. In North Carolina, winegrowing is being considered for farm diversification and as a possible alternative crop to tobacco. New growers, especially in the southeastern United States, are planting in areas without viticultural traditions. They are faced with a wide range of unusual problems relating to the climate and the large reservoir of endemic pathogens and pests flourishing on the abundant population of wild vines (McGrew, 1982). With the warmer climate, high humidity, and in particular, the potential for frequent rainfall when fruit are nearing harvest, bitter rot is one of the diseases winegrowers must be prepared to manage.

However, bitter rot challenges both growers and researchers alike because literature on the disease and the causal organism, *G. uvicola,* is lacking and outdated. Early research focused only on its identification and control (Clayton, 1975; Critopoulos, 1961; Kato et al., 1978; Luttrell, 1953; McGrew, 1977; Prakash et al., 1974; Reddy &

Reddy, 1983). Programs for the management of bitter rot based on these studies have been most effective when a combination of sanitation measures, canopy management, and applications of protectant fungicides every 10 to 14 days from bloom to harvest are used. Since infections by *G. uvicola* can remain latent until late in the season (Kummuang et al., 1996b), novice growers, weighing the economic cost, may decide against additional fungicide applications on the symptom-free fruit in anticipation of harvest, resulting in disastrous late season losses to bitter rot. Conversely, when more experienced growers expect rain or severe weather events and are juggling tight harvest schedules, they may feel pressured to avoid the disease by harvesting earlier and before the sugar and acidity balance is optimum. McGrew (1982) suggested that this early harvest may account for the reputation of high acidity in wines produced in the eastern United States

The decisions that growers face in managing bitter rot could be greatly enhanced by a better understanding of the biology and epidemiology of the disease. Unfortunately, the few available epidemiological studies have been conducted primarily on grape species other than *V. vinifera* (Kao et al., 1990; Kummuang et al., 1996a; Kummuang et al., 1996b), which is of most importance to the wine industry worldwide. Additionally, studies on the susceptibility of *V. vinifera* to bitter rot used cultivars popular in countries other than the United States (Critopoulos, 1961; Kato et al., 1978; Luttrell, 1953; Prakash et al., 1974; Reddy & Reddy, 1983).

Consequently, the objective of this study was to better understand the epidemiology of bitter rot in winegrapes by (i) examining the variation in aggressiveness among isolates from *V. vinifera* and *V. rotundifolia*, (ii) distinguishing the period of fruit susceptibility in *V. vinifera*, (iii) characterizing the relative susceptibility of winegrape cultivars to bitter rot, and (iv) determining the influence of temperature and duration of wetness on infection of *V. vinifera* fruit.

MATERIALS AND METHODS

3.1 Isolation technique and isolate identification. Fruit selected for isolation were surface disinfested with 0.525% NaOCl for 30 s and allowed to dry on paper towels in a laminar flow hood. Isolations were made by excising a section of the infected area and placing it on potato dextrose agar (PDA) acidified with 50% lactic acid. Culture dishes were incubated in a growth chamber at 26°C with continuous light for 7 to 14 days until stored at 5°C as mycelial mats desiccated on filter paper.

Prior to storage, each putative isolate of *G. uvicola* was compared to published descriptions of mycelial growth, acervuli, and conidia (Sutton & Gibson, 1977). Additionally, Koch's postulates were performed with each isolate. Uninfected, detached *V. vinifera* and *V. rotundifolia* fruit were surface-disinfested, placed in a 30.2 cm x 12.4 cm x 7.6 cm plastic moisture chamber (Pioneer Packaging, Dixon, KY) lined with wet paper towels, and inoculated with a drop of spore suspension (10^5 spores/ml). The fruit were incubated at 26°C with continuous light until symptoms developed. Symptoms and signs from inoculated fruit were identical to those on the diseased fruit collected, and cultures typical of *G. uvicola* were recovered upon reisolation.

Eighteen isolates of *G. uvicola* were obtained during 2002 and 2003 from infected fruit from Mississippi, Maryland, and five locations in North Carolina (Table 1). Isolate WB5, used throughout this study unless otherwise stated, was obtained in 2002 from *V. vinifera* 'Chardonnay' at Westbend Vineyards in Forsyth Co., NC.

3.2 Inoculum preparation. Spore suspensions for the experiments were prepared from 20 to 30-day-old cultures of *G. uvicola* growing on PDA. Conidia were released by flooding a culture with sterile distilled water and scraping the surface with a sterile dissecting needle. The suspension was then filtered through a double-layer of sterile cheesecloth into a flask of sterile distilled water, and the spore concentration was adjusted to 10^5 spores/ml with the aid of a haemocytometer.

3.3 Variation in aggressiveness among isolates from *V. vinifera* and *V. rotundifolia*. Ten isolates of *G. uvicola* were selected for studying variation among isolates. Four isolates, designated as HCMD1, HCMD5, WB4, and WB5, were obtained from *V. vinifera* fruit whereas five isolates, designated as WC3, WC5, BC2, and MS2, were obtained from *V. rotundifolia* fruit. Isolate SH was obtained from the French-American hybrid Chambourcin (Table 1).

Fruit of *V. vinifera* 'Chardonnay' were harvested at ~ 19°Brix from vines grown at the North Carolina Department of Agriculture & Consumer Services Upper Piedmont Research Station (UPRS) in Rockingham Co., NC, and the Bloodworth Vineyard in

Orange Co., NC. The soluble solids content, measured in °Brix, was obtained by averaging the juice of 10 individual fruit for each cultivar using a 0-32°Brix Fisherbrand handheld refractometer (Fisher Scientific International).

The fruit were surface disinfested with 0.525% NaOCl for 30 s, rinsed with distilled water, and allowed to dry overnight in a laminar flow hood. Individual fruit were clipped at the pedicel to avoid injury and placed on wire mesh racks in plastic moisture chambers lined with paper towels moistened by 150 ml of distilled water. The racks were cut to fit from hot-dipped galvanized woven wire mesh with 1.7 cm x 1.7 cm openings. For each isolate, 10 detached fruit from each of four Chardonnay clusters were arbitrarily selected and atomized with a conidial suspension (10^5 spores/ml) using a SPI Crown Portable Spra-Tool (Aervoe Industries Incorporated, Gardnerville, NV). Ten fruit from each of four clusters were also included in the study as a noninoculated control. The plastic chambers were sealed with masking tape to maintain free moisture on the fruit surface during incubation. The fruit were then incubated in growth chambers at 26°C under continuous light for 7 days before disease severity was evaluated.

Disease severity was evaluated using the following scale: 0 = no infection, 1 = 1 to 5% fruit surface with visible symptoms, 2 = 6 to 15%, 3 = 16 to 50%, and 4 ≥ 51% (Fig. 1). Presence of acervuli and secondary contaminants were also noted. The study was repeated two times.

Data were analyzed using the SAS System for Windows, Release 8.02 (SAS Institute, Inc., Cary, NC). For the purpose of statistical analysis, disease severity was averaged over each of the four clusters and used as the response variable. An analysis of variance (ANOVA) was conducted with a general linear model procedure (PROC GLM), and hypotheses were tested at $\alpha = 0.05$ level of significance. The separation of means by isolate was achieved using the Waller-Duncan *k*-ratio *t* test where $k = 100$, and orthogonal contrasts on host species were examined using a CONTRAST statement.

3.4 Period of fruit susceptibility in *V. vinifera*. Field studies were conducted during the 2003 and 2004 growing seasons. *V. vinifera* ‘Chardonnay’ and ‘Cabernet Franc’ vines at the UPRS in Rockingham Co., NC, and *V. vinifera* ‘Merlot’ vines at Iron Gate Vineyards in Alamance Co., NC, were planted in 2000 on a 3 m x 2 m spacing and trained using a bilateral cordon trellising system with vertical shoot positioning. A completely randomized experimental design with four replications was used for Chardonnay and Cabernet Franc and in 2004 for Merlot. A factorial experimental design was used in 2003 for Merlot where cane-pruning and spur-pruning each had two replications.

Fruit of Chardonnay, Cabernet Franc, and Merlot were atomized biweekly with a conidial suspension of *G. uvicola* (10^5 spores/ml) starting from bloom until 2 weeks before harvest. Chardonnay and Merlot were inoculated six times each field season, but due to differences in cultivar ripening, Cabernet Franc was inoculated seven times in both seasons. Five clusters were arbitrarily selected for each of four replications, where a cordon or whole vine was considered a replication, for every inoculation date. The intact clusters were tagged with colored vinyl slip-on plant tags (Earth Star, Inc., Winchester, KY) and atomized with a conidial suspension in the late afternoon and bagged overnight in 0.7 L and 1.8 L Fisherbrand Sterile Sampling Bags with Flat-Wire Closures (Fisher Scientific International). Prior to sealing, the bags were misted inside with sterile distilled water to maintain the free moisture on the fruit. The bags were removed early the next morning before temperatures rose. Vines selected for inoculation were flagged and not sprayed with fungicides for 2 weeks prior to inoculation and for another 2 weeks following inoculation. Inoculation dates were as follows: 29 May (bloom), 10 and 25 (bunch closing) June, 9 and 23 (véraison for Merlot) July, and 5 (véraison for Chardonnay and Cabernet Franc) and 20 August for 2003; and 20 May (bloom), 3 and 17 (bunch closing) June, 1, 14 (véraison for Merlot), 29 (véraison for Chardonnay and Cabernet Franc) July, and 12 August (Cabernet Franc only) for 2004.

A captan-based spray program was applied throughout the season (Table 2). Fungicide sprays were made with a Stihl gasoline-powered mist blower at the equivalent of 935.4 L/ha. The Chardonnay and Cabernet Franc vines were covered with bird netting on 18 August 2003 and 19 August 2003, respectively, and for both cultivars on 7 July 2004. Disease incidence was evaluated at harvest by counting the number of symptomatic berries and total number of berries per cluster. Fruit were harvested at ≥18°Brix on 25 August 2003 and 18 August 2004 for Chardonnay, on 3 September 2003 and 27 August 2004 for Cabernet Franc, and on 20 August 2003 and 12 August 2004 for Merlot.

Weather data were obtained through the North Carolina Climate Retrieval and Observations Network of the Southeast Database (NC CRONOS Database, web address: http://www.nc-climate.ncsu.edu/cronos/). Daily average temperature, average relative humidity, and precipitation data were obtained for the Rockingham Co. vineyard from the

UPRS site, approximately 500 m from the vineyard, and for the Alamance Co. vineyard from the Burlington Alamance Airport, approximately 22 km away. In addition, leaf wetness and temperature inside one inoculation bag were monitored overnight using Watchdog Leaf Wetness and Temperature Loggers (Spectrum Technologies, Inc., Plainfield, IL) for every inoculation date in both vineyards in 2003 and 2004.

For the purpose of statistical analysis, disease incidence was averaged over the five clusters of each cordon or whole vine (i.e. replication) and used as the response variable. An analysis of variance (ANOVA) was conducted with a general linear model procedure (PROC GLM), and the separation of means was achieved using the Waller-Duncan k-ratio t test where $k = 100$. The three cultivars were analyzed separately due to the differences in location and experimental design. The study conducted in Merlot was analyzed for the two seasons by using only the data collected from spur-pruned treatments in 2003 and all of the data collected in 2004, since all vines were spur-pruned that year. Regression analysis was conducted using the incidence data for each cultivar to obtain the best model using PROC REG in SAS.

3.5 Relative susceptibility of cultivars to bitter rot. Fruit to be tested for susceptibility to bitter rot were harvested primarily from the Bloodworth Vineyard in Orange Co., NC. Due to limited availability in 2003, fruit of the following *V. vinifera* cultivars were obtained from other sources: Cabernet Sauvignon and Cabernet Franc from Round Peak Vineyards in Surry Co., NC; Riesling from Westbend Vineyards in Forsyth Co., NC; Merlot from Iron Gate Vineyards in Alamance Co., NC; and

Sangiovese, Cabernet Franc, Syrah, Tempranillo, and Chardonnay from the UPRS in Rockingham Co., NC. Fruit of 30 and 33 grape cultivars and selections were harvested at ≥16°Brix in 2003 and 2004, respectively (Table 3).

The fruit were surface disinfested with 0.525% NaOCl for 30 s, rinsed with distilled water, and allowed to dry overnight in a laminar flow hood. Individual fruit were clipped at the pedicel to avoid injury and placed on mesh racks in plastic moisture chambers as previously described. For each of four clusters per cultivar, 20 detached fruit were atomized with a conidial suspension (10^5 spores/ml), and five fruit per cluster were used as a noninoculated control. The plastic chambers were sealed with masking tape to maintain moisture. Fruit were then incubated in growth chambers at 26°C under continuous light for 7 days before disease severity was evaluated, using the same scale described above.

Each cultivar or selection was repeated two times during the study with the exception of *V. vinifera* ‘Cabernet Franc,’ which was repeated three times, and the following cultivars were inoculated only once due to lack of availability: *V. vinifera* ‘Riesling,’ ‘Sangiovese,’ ‘JB97-8-0-7,’ ‘Petite Sirah,’ ‘JB96-14-7-27,’ ‘Semillon,’ ‘Barbera,’ ‘JB96-14-7-12,’ ‘Perlette,’ ‘JB94-13-2-26,’ ‘JB97-8-6-54,’ ‘Touriga Nacional,’ and *V. labrusca* ‘Sunbelt.’ There were 38 different cultivars and selections included in the study in total.

For the purpose of statistical analysis, disease severity was averaged over each cluster and used as the response variable. An analysis of variance (ANOVA) was conducted with a general linear model procedure (PROC GLM). Effects of interest included species, time of ripening, soluble solids (i.e. Brix), and fruit color (Table 3), and the separation of these means was achieved using the Waller-Duncan *k*-ratio *t* test where $k = 100$.

3.6 Influence of temperature and duration of wetness on infection. Fruit of *V. vinifera* 'Chardonnay,' 'Cabernet Franc,' and 'Cabernet Sauvignon' were harvested at ≥18°Brix in 2003 and 2004. Clusters of Chardonnay and Cabernet Franc were obtained from the UPRS in Rockingham Co., NC, in 2003 and 2004. Additional Chardonnay fruit were obtained in 2003 from Laurel Gray Vineyards in Yadkin Co., NC. Clusters of Cabernet Sauvignon were obtained from the Bloodworth Vineyard in Orange Co., NC, in 2003 and 2004. Additional Cabernet Franc and Cabernet Sauvignon fruit were obtained from Round Peak Vineyards in Surry Co., NC, in 2003.

Fruit were surface disinfested with 0.0525% NaOCl for 30 s, rinsed with distilled water, and allowed to dry overnight in a laminar flow hood. Individual fruit were clipped at the pedicel to avoid injury and placed on mesh racks in plastic moisture chambers, as previously described. The detached fruit were atomized with a conidial suspension (10^5 spores/ml).

The experimental design was a split-plot with the whole-plot factor of temperature at levels of 14, 18, 22, 26, and 30°C in growth chambers and a sub-plot factor of duration of wetness at levels of 6, 12, 18, and 24 hours. There were three replications of 10 fruit per temperature and wetness combination, and the study was repeated three times for each cultivar. After completion of each wetting duration, further infection was stopped by immersing fruit in 0.525% NaOCl for 30 s. Fruit were then rinsed with distilled water, allowed to dry, and incubated at 26°C under continuous light on mesh racks in dry plastic chambers sealed with masking tape until evaluated 9 to 10 days later for disease severity with the same scale described above.

For statistical analysis, disease severity was averaged over each replication (i.e. 10 fruit for each temperature and wetness combination) and used as the response variable. An analysis of variance (ANOVA) was conducted with a general linear model procedure (PROC GLM), and the separation of these means were achieved using the Waller-Duncan *k*-ratio *t* test where *k* = 100.

RESULTS

4.1 Variation in aggressiveness among isolates from *V. vinifera* and *V. rotundifolia*. Isolates of *G. uvicola* differed in aggressiveness when tested on the cultivar Chardonnay (*P* = 0.0001). Isolates HCMD1 and HCMD5, obtained from a *V. vinifera* vineyard in Maryland, were the most aggressive with mean severities of ~ 3.0 (Fig. 2). Isolate WB5, obtained from a *V. vinifera* vineyard in North Carolina and used in all additional studies, was moderately aggressive with a mean disease severity of 2.1, which was not significantly different from three other isolates collected in North Carolina (i.e. WB4, WC3, WC5). Disease severity on the Chardonnay fruit inoculated with isolates collected from *V. vinifera* was significantly higher than with isolates collected from *V.*

rotundifolia (P = 0.0001). Isolate SH, obtained from the French-American hybrid 'Chambourcin,' was less aggressive than isolates obtained from *V. vinifera* (P = 0.0001) or *V. rotundifolia* (P = 0.0009).

4.2 Period of fruit susceptibility in *V. vinifera*. When data from both years were combined, there were strong interactions for year x inoculation date with P-values of 0.0024, 0.0001, and 0.0011 for Chardonnay, Cabernet Franc, and Merlot, respectively. Because the strong interaction implied inoculation date effects were not consistent among cultivars and between years, the three cultivars were analyzed separately for each year. In 2003 Chardonnay and Cabernet Franc experienced heavy bird feeding, resulting in fewer berries per cluster.

The inoculation date main effect for disease incidence in Chardonnay fruit was significant for 2003 (P = 0.0472) and 2004 (P = 0.0001). The susceptibility of Chardonnay fruit increased from bloom until véraison in 2003 and from bloom until 2 weeks before véraison in 2004 (Fig. 3). Fruit were most susceptible when clusters reached véraison in 2003. In 2004 disease incidence was significantly higher 6 to 8 weeks after bloom than at any other time during the season.

Similar results were observed in Cabernet Franc where the inoculation date main effect was significant in 2003 (P = 0.0001) and 2004 (P = 0.0001). Following inoculation, the highest percent of bitter rot infection occurred 8 to 10 weeks and 4 to 6 weeks after bloom in 2003 and 2004, respectively (Fig. 4).

Fruit of Merlot increased in susceptibility to bitter rot from bloom until véraison in 2003 and until 2 weeks before véraison in 2004 (Fig. 5). Inoculation date main effects were significant in both 2003 (P = 0.0021) and 2004 (P = 0.0003). Several of the vines inoculated in the Merlot planting were discovered later to be *V. vinifera* 'Sauvignon Blanc,' resulting in three missing data points in 2003. The incidence of bitter rot was higher in cane-pruned vines (mean = 0.64, standard deviation = 0.26) than vines that were spur-pruned (mean = 0.47, standard deviation = 0.32) in 2003. Fruit infection of Merlot was highest when clusters were inoculated 6 to 10 weeks and 4 to 8 weeks after bloom in 2003 and 2004, respectively. Data were also examined after arcsine-square root transformation, and the subsequent analysis yielded similar results.

The best model for predicting susceptibility of fruit to bitter rot for the three cultivars in either year was $\text{Dis}(y) = b_0 + b_1(\text{date}) + b_2(\text{date}^2) + b_3(\text{date}^3)$ (Fig. 3, 4, 5). This third-order polynomial model predicted maximum disease incidence from inoculations made 8 to 9 weeks after bloom in 2003, although the model could not predict a clear maximum for Chardonnay, and from inoculations made 6 to 7 weeks after bloom in 2004. These results from the model, which had high R^2 values ranging from 0.85 to 0.99, are consistent with field observations.

Weather during both seasons was conducive for bitter rot development following inoculation. The 2003 growing season was very wet with 61.9 and 64.2 cm total precipitation when compared to 2004 with just 35.9 and 34.4 cm for Rockingham Co. and Alamance Co., respectively (NC CRONOS Database, Table 4). The vineyards in Rockingham Co. and Alamance Co. also experienced 22 and 8 more days, respectively, of precipitation in 2003 than in 2004. Monthly average temperatures ranged from 18.1 to

25.0°C and from 15.1 to 25.1°C in 2003 and 2004, respectively.

Temperatures in the inoculation bags in the Alamance Co. vineyard were 22.3 ± 5°C (standard deviation) and 24.8 ± 5°C with 9 and 14 hours of wetness in 2003 and 2004, respectively. No leaf wetness data were obtained for the 29 May 2003 inoculation because the logger was placed in an improperly sealed bag. Temperatures in the inoculation bags in the Rockingham Co. vineyard were 23.5 ± 5°C in 2003 and 23.7 ± 4°C in 2004 with 15 hours of wetness in both years. An exception was the 29 May 2003 inoculation, which had an average of 14.7 ± 4°C with 11 hours of wetness. Data were missing for both vineyards for 3 June 2004 because the sensors in the logger were flooded prior to the inoculation.

4.3 Relative susceptibility of cultivars to bitter rot. A wide range of susceptibility was observed among the cultivars and selections included in the study (Fig. 6). The mean disease severity of over 20% of the cultivars and selections tested (n = 38) fell between 2.0 and 2.5 on the disease severity scale, and over 50% of the cultivars and selections tested had disease severity means between 1.0 and 2.5.

The species main effect on disease severity was significant (*P* = 0.0001). Fruit of *V. vinifera* (n = 23) and other species (n = 10) were significantly more susceptible to bitter rot than French-American hybrids (n = 5) (Table 3, Fig. 6). Mean disease severities of *V. vinifera*, other species, and French-American hybrids were 2.0, 1.8, and 0.8, respectively. Cultivars Petit Sirah, JB97-8-0-7, MissBlanc, Roussanne, Mourvédre, and

Petit Verdot were among the most susceptible to bitter rot whereas Cynthiana, Arkansas 1271, Traminette, Chardonel, and Riesling were among the most resistant. *V. aestivalis* 'Cynthiana' was the only resistant cultivar. The inoculated fruit of Cynthiana remained free of bitter rot symptoms for up to 14 days at 26°C. Secondary contamination in the noninoculated controls of each cultivar or selection was less than 5% except in JB97-8-6-54 and one replication of NC74CO4482, where ~ 20% of the fruit experienced contamination.

The effects of fruit color and time of ripening were not significant with P-values of 0.2428 and 0.4799, respectively. The soluble solids main effect was also not significant ($P = 0.1839$) although the least infection occurred in fruit of 15 and 16°Brix. Interactions between fruit color and time of ripening as well as species and time of ripening were significant with *P*-values of 0.0192 and 0.0015, respectively. These results are consistent since cultivars with white fruit generally ripen earlier than cultivars with red fruit, and véraison occurs at different times for different cultivars.

4.4 Influence of temperature and duration of wetness on infection. When data from all three cultivars were combined, the main effects of temperature ($P = 0.0001$) and duration of wetness ($P = 0.0001$) were significant. Bitter rot infection was most severe at temperatures of 22 and 26°C and at 6 and 12 hours of wetness (Fig. 7). The best models for bitter rot infection of fruit was $\mathrm{Dis}(y) = b_0 + b_1(\mathrm{temp}) + b_2(\mathrm{temp}^2) + b_3(\mathrm{temp}^3)$ for temperature and $\mathrm{Dis}(y) = b_0 + b_1(\mathrm{wet}) + b_2(\mathrm{wet}^2) + b_3(\mathrm{wet}^3)$ for wetness (Fig. 7). These third-order polynomial models predicted the optimal conditions for infection of fruit by

G. uvicola to be a temperature of 23.7°C and 9 hours of wetness with R^2 values of 0.99 for both effects. The interaction of temperature x duration of wetness was also significant (P = 0.001) with the highest infections occurring at 26°C with 12 hours of wetness and 22°C with 6 hours of wetness (Fig. 8).

The analysis of variance indicated a strong cultivar main effect (P = 0.0028). Higher infection occurred on Chardonnay with a mean disease severity of 2.36 than Cabernet Sauvignon or Cabernet Franc. Since all interactions of cultivar with temperature and duration of wetness were also significant, the three *V. vinifera* cultivars were further analyzed separately. Temperature and duration of wetness main effects remained significant even when cultivars were separated. The temperature x duration of wetness interaction was significant for Chardonnay and Cabernet Sauvignon with P-values of 0.0013 and 0.0174, respectively, although not significant for Cabernet Franc (P = 0.0651). No replication main effect or interaction was significant in any of the analyses.

DISCUSSION

Fruit of *V. vinifera* ‘Chardonnay,’ ‘Cabernet Franc,’ and ‘Merlot’ were susceptible to infection by *G. uvicola* from bloom until harvest. Previous studies with other *Vitis* species (Kao et al., 1990; Ridings & Clayton, 1970) found that fruit were susceptible prior to véraison but did not extend their studies beyond that stage. The relationship between disease incidence and inoculation date was nonlinear, and the model

[Dis (y) = $b_0 + b_1(\text{date}) + b_2(\text{date}^2) + b_3(\text{date}^3)$] predicted fruit were most susceptible 8 to 9 weeks after bloom in 2003 and 6 to 7 weeks after bloom in 2004. This differs from black rot, caused by *Guignardia bidwellii*, where fruit are most susceptible 3 to 5 weeks after bloom with decreased susceptibility until 6 to 7 weeks postbloom (Hoffman et al., 2002).

Wounding was not a requirement for infection of *V. vinifera* fruit as previous research in other species suggested (Kummuang et al., 1996a; Ridings & Clayton, 1970). The inoculated *V. vinifera* fruit were not russetted, which has also been associated with early infection of *V. rotundifolia* (Kummuang et al., 1996b). Typical bitter rot symptoms did not develop until later in the season, supporting previous work that symptom expression occurs when soluble solids have increased (Kao et al., 1990). Since sporulation on the fruit was observed only toward the end of season, it is likely that excess rainfall, leading to splitting and cracking of mature fruit, and damage from bird feeding accounted primarily for increased secondary infection, resulting in the highest incidence in 2003 occurring 2 weeks later than in 2004. The Cabernet Franc fruit remained on the vines for 9 days longer than the Chardonnay fruit in both years. This additional exposure to secondary cycles of inoculum may account for the increased incidence in the more resistant Cabernet Franc cultivar at harvest in the same location.

Disease incidence was slightly higher in cane-pruned vines of Merlot than spur-pruned vines in 2003. These results reflect only one growing season, and further studies using the same factorial experimental design would be required to determine whether pruning method is a consistent and significant factor affecting disease incidence.

The optimal conditions for infection of *V. vinifera* fruit by *G. uvicola*, determined by fitting third-order polynomial models to the data, were a temperature of 23.7°C and 9 hours of wetness. This temperature is slightly lower than previously found when detached fruit of *V. rotundifolia* (Ridings & Clayton, 1970) and *V. labrusca* (Kao et al., 1990) were inoculated. Because infection of fruit can occur with as few as 9 hours of wetness, it is important for growers to maintain a protectant fungicide program because environmental conditions are conducive for infection of fruit by *G. uvicola* throughout the season in areas with climates similar to those in North Carolina. It is also important to minimize duration of wetting periods by leaf pulling and pruning to maintain an open, aerated canopy.

Isolates of *G. uvicola* obtained from *V. vinifera* were significantly more aggressive on Chardonnay fruit than isolates obtained from *V. rotundifolia*. These results may explain why Clayton (1975) stated that losses from bitter rot in *V. vinifera* in the field may be higher than in *V. rotundifolia*. The most aggressive isolates tested were obtained from Cabernet Franc fruit from a Maryland vineyard with a chronic bitter rot problem. In our studies, Cabernet Franc was more resistant to bitter rot than Chardonnay. Thus, *Vitis* spp. and cultivar may be factors selecting for more aggressive isolates. Further studies to confirm this hypothesis would require examining a larger collection of *G. uvicola* isolates and possibly characterizing the diversity of the population through molecular analyses.

A wide range of susceptibility to bitter rot was observed among the 38 cultivars and selections tested. Fruit of *V. vinifera* were significantly more susceptible than French-

American hybrids, suggesting that interspecific hybrids can inherit increased resistance from their American parent and that the bitter rot fungus is endemic to North America.

Cultivars Cabernet Franc, Cabernet Sauvignon, and Seyval had similar susceptibility to infection by *G. uvicola* as to other fruit rots, whereas Merlot and Sauvignon Blanc were more resistant to infection by the bitter rot pathogen than reported to other fruit rot fungi (Wolf & Poling, 1996). The susceptibility of Cynthiana, Viognier, Chardonnay, Chambourcin, Traminette, and Vidal Blanc cultivars to the bitter rot fungus is similar to their susceptibility to black rot, Phomopsis, and Botrytis bunch rot pathogens (Southeastern Grape IPM, 2004).

Little was known of the susceptibility of winegrape cultivars to infection by *G. uvicola*. In addition to aiding plant breeders with making their selections, the results of this study can impact growers in challenging production areas by allowing them to formulate more informed decisions about which cultivars to establish in their vineyard. Even if growers are reluctant to switch from a popular cultivar, whose fruit and juice constitute the major components in producing a varietal wine, these results can affect their selections for grapes used for blending purposes. The utilization of less susceptible cultivars in combination with good canopy management and regular fungicide applications has the potential to provide reliable control of bitter rot even in production areas with warm, humid climates. In addition, the resistance of *V. aestivalis* ‘Cynthiana’ to bitter rot and other fruit rots strengthens the case for the cultivar’s potential for organic wine production in the southeastern United States.

This increased understanding of the period of fruit susceptibility has practical implications for both epidemiology and management of the disease. Since fruit of *V. vinifera* are susceptible throughout the season, fungicide sprays from bloom until véraison are critical, and omission or misapplication of any single spray can result in poor fruit quality and yield loss due to bitter rot. Although rainfall in early summer has been correlated with high incidence at harvest (Kummuang et al., 1996b), our results suggest that in production areas with high potential for precipitation near harvest, growers should be especially vigilant in management of the disease prior to exposure to secondary cycles as well. Although the relationship between disease incidence and inoculation date gave high R^2 values, more practical models for disease forecasting should incorporate other factors including inoculum availability, cultivar, temperature, rainfall and leaf wetness, canopy training system, and differences in isolate aggressiveness.

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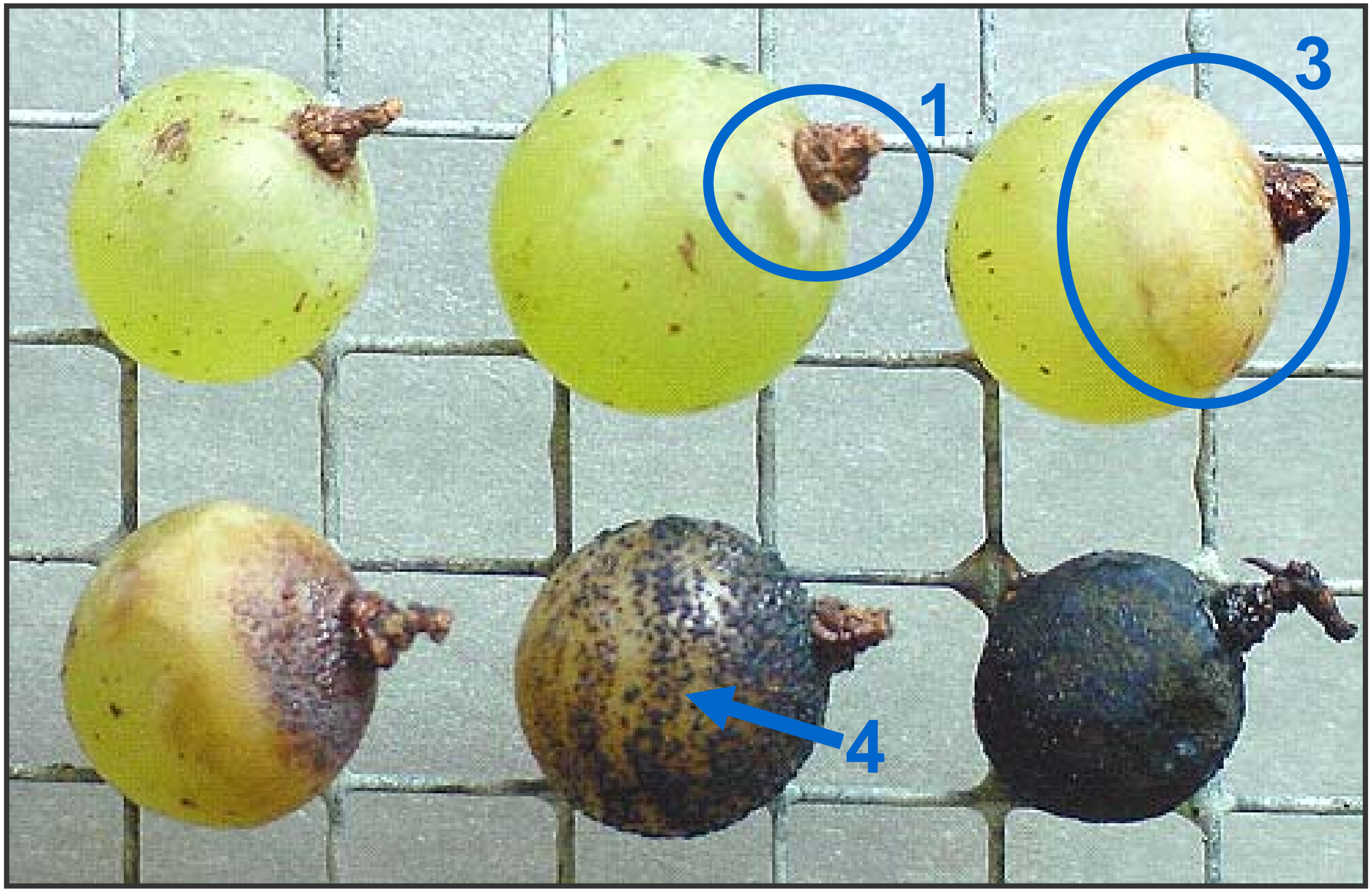

Figure 1. Bitter rot symptom development on inoculated detached *Vitis vinifera* fruit incubated at 26°C under continuous light for 7 days. Disease severity was evaluated 7 days after inoculation, using the following scale: 0 = no infection, 1 = 1 to 5% fruit surface with visible symptoms, 2 = 6 to 15%, 3 = 16 to 50%, and 4 ≥ 51%. Numbers indicate disease severity ratings for the detached fruit.

Table 1. Isolates of *G. uvicola* obtained from *Vitis* spp. in 2002 and 2003.

Isolate Designation	Geographical Origin	Source		Year Collected
		Host species	**Cultivar**	
HCMD 1	Anne Arundel Co., MD	*V. vinifera*	Cabernet Franc	2003
HCMD 3	Anne Arundel Co., MD	*V. vinifera*	Cabernet Franc	2003
HCMD 5	Anne Arundel Co., MD	*V. vinifera*	Cabernet Franc	2003
HCMD 6	Anne Arundel Co., MD	*V. vinifera*	Cabernet Franc	2003
WB 4	Forsyth Co., NC	*V. vinifera*	Chardonnay	2002
WB 5	Forsyth Co., NC	*V. vinifera*	Chardonnay	2002
WC 2	Wake Co., NC	*V. rotundifolia*	Nesbitt	2002
WC 3	Wake Co., NC	*V. rotundifolia*	Nesbitt	2002
WC 4	Wake Co., NC	*V. rotundifolia*	Nesbitt	2002
WC 5	Wake Co., NC	*V. rotundifolia*	Nesbitt	2002
WC 6	Wake Co., NC	*V. rotundifolia*	Nesbitt	2002
BC 1	Bladen Co., NC	*V. rotundifolia*	Carlos	2002
BC 2	Bladen Co., NC	*V. rotundifolia*	Carlos	2002
MS 1	Pearl River Co., MS	*V. rotundifolia*	Carlos	2002
MS 2	Pearl River Co., MS	*V. rotundifolia*	Carlos	2002
HCRS 1	New Hanover Co., NC	*V. rotundifolia*	Carlos	2002
HCRS 2	New Hanover Co., NC	*V. rotundifolia*	Fry	2002
SH	Chatham Co., NC	French-American hybrid	Chambourcin	2002

Table 2. Pesticide spray program for the vineyards in Rockingham Co. and Alamance Co., NC, for 2003 and 2004.

Stage of *Vitis vinifera*	Date of Application		Pesticide (Trade Name) Formulation[a]	Rate (per hectare)[b]
	2003 Growing Season	2004 Growing Season		
Prebloom	21 May 2003	13 May 2004	mancozeb 75DF sulfur	3.36 kg 4.48 kg
Bloom	29 May 2003	20 May 2004	phosmet (Imidan) 70WP fenhexamid (Elevate) 50 WDG	1.49 kg 1.12 kg
Postbloom	10 June 2003	3 June 2004	mancozeb 75DF myclobutanil (Nova) 40W potassium phosphite (ProPhyt)	3.36 kg 0.29 L 0.03 L
Bunch closing	25 June 2003	17 June 2004	phosmet (Imidan) 70WP fenhexamid (Elevate) 50 WDG captan 50 WP myclobutanil (Nova) 40W	1.49 kg 1.12 kg 3.36 kg 0.29 L
(cover spray)	9 July 2003	1 July 2004	captan 50 WP myclobutanil (Nova) 40W	3.36 kg 0.29 L
(Véraison for Merlot)	23 July 2003	14 July 2004	captan 50WP cyprodinil (Vanguard) 75 WG	3.36 kg 0.73 L
(Véraison for Chardonnay and Cabernet Franc)	5 August 2003	29 July 2004	captan 50WP	3.36 kg
Preharvest	20 August 2003	12 August 2004	captan 50WP	3.36 kg

[a] Carbaryl (Sevin) 80WP was applied at a rate of 1.40 kg/ha, replacing phosmet at bunch closing on 17 June 2004.

[b] Pesticide sprays were made with a Stihl gasoline-powered mist blower at the equivalent of 935.4 L/ha.

Table 3. Cultivars and selections of *Vitis* spp. evaluated for susceptibility to infection by *G. uvicola*.

Cultivar[a]	**Species**[b]	**Time of Ripening**[c]	**Fruit Color**	**Year(s) Collected**
Arkansas 1271	*V. vinifera*	Late	Red	2003, 2004
Barbera	*V. vinifera*	Midseason	Red	2004
Cabernet Franc	*V. vinifera*	Late	Red	2003, 2004
Cabernet Sauvignon	*V. vinifera*	Late	Red	2003, 2004
Chambourcin	French-American hybrid	Late	Red	2003, 2004
Charbono	*V. vinifera*	Late	Red	2003, 2004
Chardonel	French-American hybrid	Early	White	2003, 2004
Chardonnay	*V. vinifera*	Early	White	2003, 2004
Cynthiana (Norton)	*V. aestivalis*	Late	Red	2003, 2004
JB94-13-2-26	Other	Midseason	White	2004
JB96-11-94	Other	Midseason	White	2003, 2004
JB96-14-7-12	Other	Late	Red	2004
JB96-14-7-27	Other	Late	White	2004
JB97-8-0-7	Other	Late	White	2003
JB97-8-6-54	Other	Late	Red	2004
Merlot	*V. vinifera*	Midseason	Red	2003, 2004
MissBlanc	Other	Late	White	2003, 2004
Mourvédre (Mataro)	*V. vinifera*	Late	Red	2003, 2004
NC74CO4482	Other	Early	Red	2003, 2004
Perlette	*V. vinifera*	Early	White	2004
Petit Sirah	*V. vinifera*	Late	Red	2003
Petit Verdot	*V. vinifera*	Late	Red	2003, 2004
Riesling	*V. vinifera*	Midseason	White	2003
Rkatsiteli	*V. vinifera*	Late	Red	2003, 2004
Roussanne	*V. vinifera*	Late	White	2003, 2004
Sangiovese	*V. vinifera*	Late	Red	2003
Sauvignon Blanc	*V. vinifera*	Midseason	White	2003, 2004
Semillon	*V. vinifera*	Early	White	2004
Seyval	French-American hybrid	Midseason	White	2003, 2004
Sunbelt	*V. labrusca*	Late	Red	2003
Syrah (Shiraz)	*V. vinifera*	Midseason	Red	2003, 2004
Tannat	*V. vinifera*	Late	Red	2003, 2004
Tempranillo (Valdepenas)	*V. vinifera*	Early	Red	2003, 2004
Tinta Cao	*V. vinifera*	Late	Red	2003, 2004
Touriga Nacional	*V. vinifera*	Late	Red	2004
Traminette	French-American hybrid	Early	White	2003, 2004
Vidal Blanc	French-American hybrid	Midseason	White	2003, 2004
Viognier	*V. vinifera*	Midseason	White	2003, 2004

[a] Popular synonyms for cultivars are indicated in parentheses.

[b] Cultivars labeled as "other" species have not been publicly released or are only of interest for breeding research purposes.

[c] Time of ripening was assigned based on literature available, date of harvest, and/or plant breeder observations.

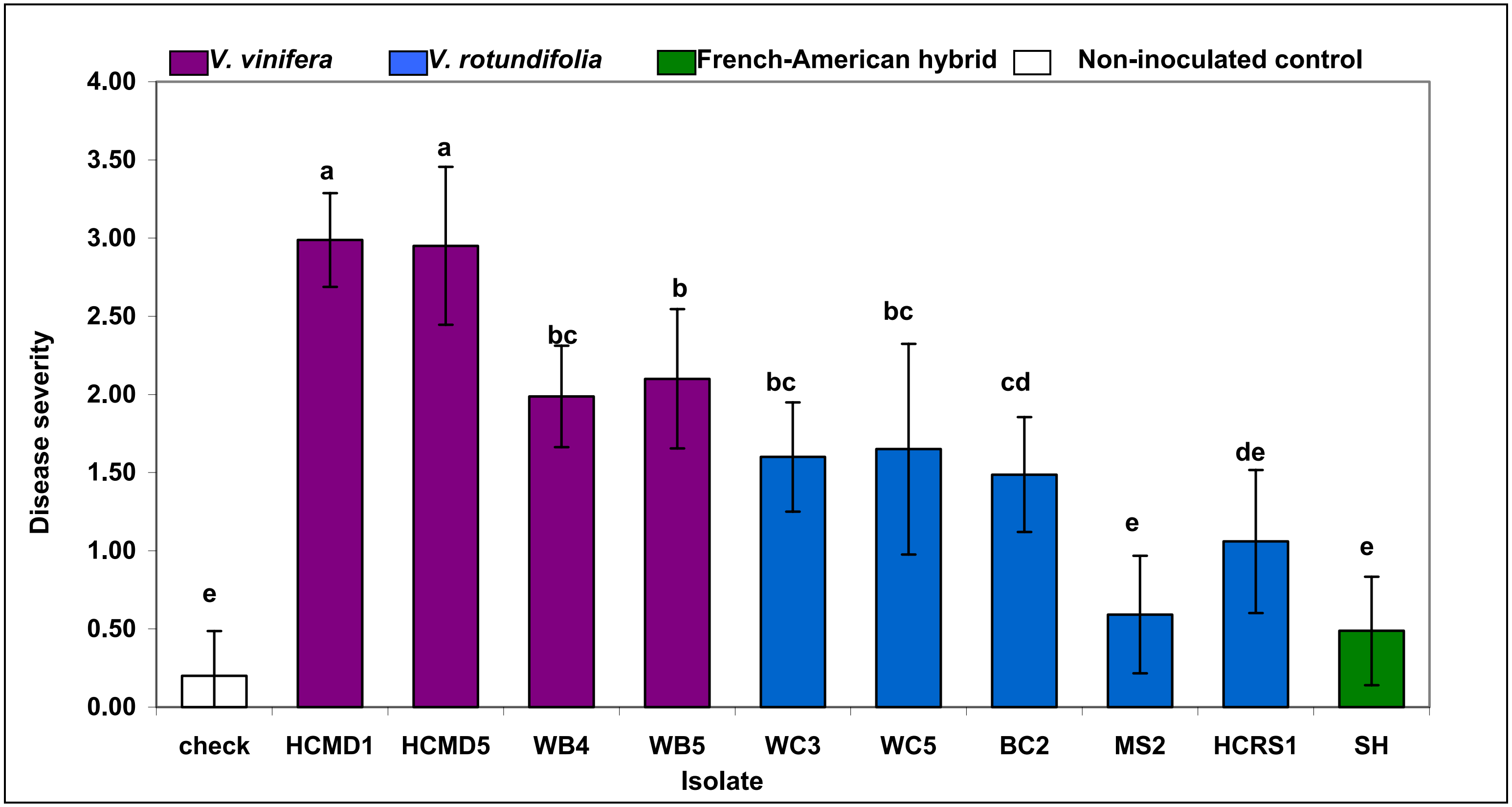

Figure 2. Variation in aggressiveness among isolates of *G. uvicola* from *Vitis* species. Bars represent the mean disease severity for each isolate, and error bars represent standard error. Disease severity on detached fruit was evaluated 7 days after inoculation, using the following scale: 0 = no infection, 1 = 1 to 5% fruit surface with visible symptoms, 2 = 6 to 15%, 3 = 16 to 50%, and 4 ≥ 51%. Bars with the same letter(s) are not significantly different according to the Waller-Duncan *k*-ratio *t* test where *k* = 100 with MSD = 0.53, R^2 = 0.72, and CV = 38.07.

Table 4. Weather data for Rockingham Co. and Alamance Co., NC, obtained from the North Carolina Climate Retrieval and Observations Network of the Southeast Database (NC CRONOS, web address: http://www.nc-climate.ncsu.edu/cronos/).

Location[a]	Year	Month[b]	Air Temperature[c] (°C)	Relative Humidity[c] (%)	Sum of Precipitation (cm)	Total Days with Precipitation
Rockingham County	2003	May	18.2	76	0.3	3
Rockingham County	2003	June	22.1	78	29.6	16
Rockingham County	2003	July	23.9	86	11.3	20
Rockingham County	2003	August	24.4	80	20.0	19
Rockingham County	2003	September	24.7	92	0.7	2
Alamance County	2003	May	18.1	75	0.5	2
Alamance County	2003	June	22.2	78	19.7	16
Alamance County	2003	July	24.5	78	22.0	18
Alamance County	2003	August	25.0	80	22.2	15
Rockingham County	2004	May	15.1	42	1.4	6
Rockingham County	2004	June	22.5	85	14.0	13
Rockingham County	2004	July	24.6	82	11.0	10
Rockingham County	2004	August	21.8	86	9.5	9
Alamance County	2004	May	24.8	70	1.3	5
Alamance County	2004	June	23.2	76	15.6	17
Alamance County	2004	July	25.1	75	14.8	15
Alamance County	2004	August	23.2	74	2.7	6

[a] Data were obtained for the Rockingham County vineyard from the North Carolina Department of Agriculture & Consumer Services Upper Piedmont Research Station site, approximately 500 m from the vineyard, and for the Alamance County vineyard from the Burlington Alamance Airport, approximately 22 km away.

[b] Data only include observations starting from the date of the first inoculation of the season through harvest at each location. First inoculation date and last harvest at each location respectively were 29 May and 3 September 2003 and 20 May and 27 August 2004 at Rockingham Co. and 29 May and 20 August 2003 and 20 May and 12 August 2004 at Alamance Co.

[c] Monthly averages were calculated from daily means for temperature and humidity at 2 m.

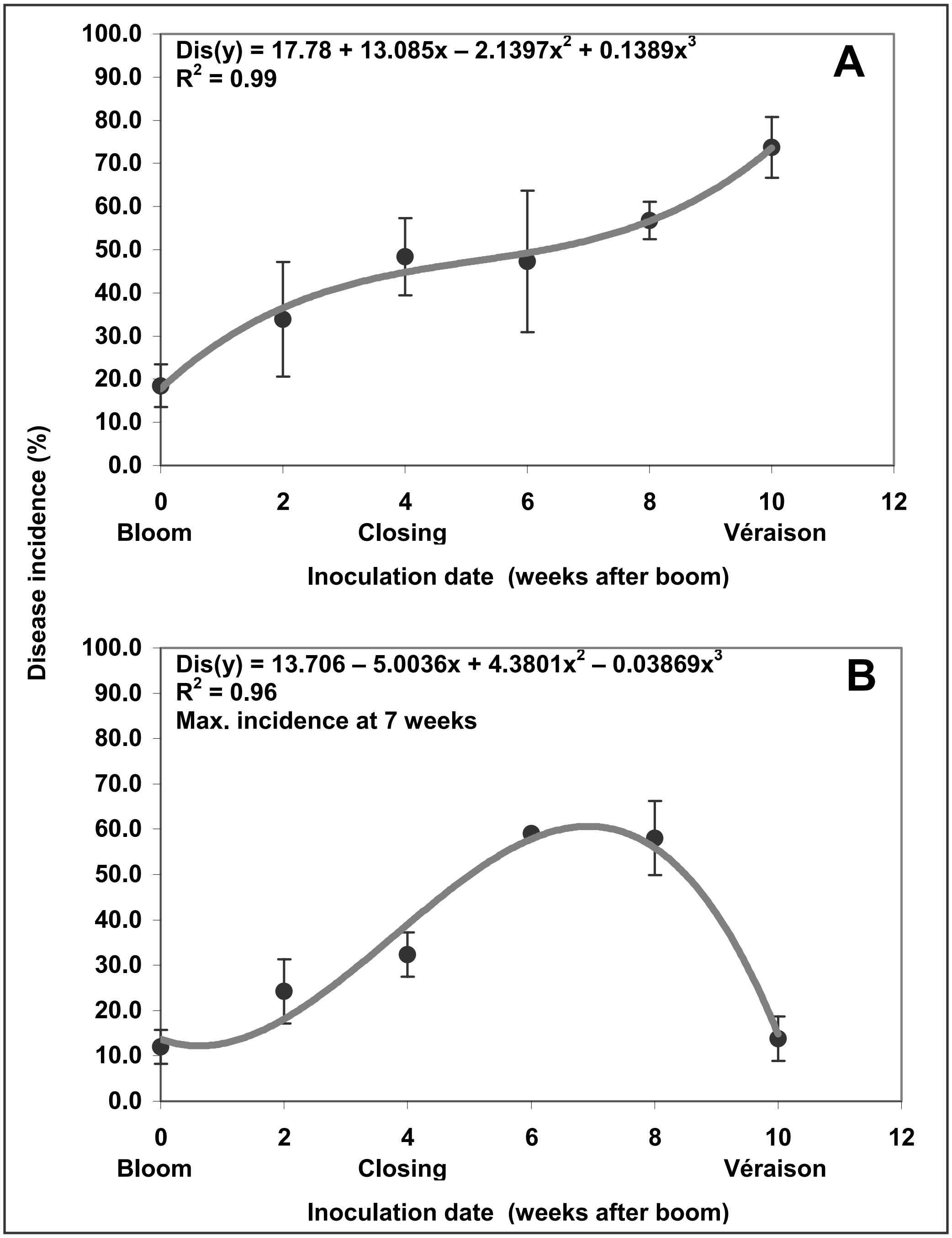

Figure 3. Relationship between disease incidence and inoculation date for Chardonnay fruit from bloom until véraison in 2003 (A) and 2004 (B). Each point represents the mean disease incidence at harvest for a whole vine or cordon (n = 4). Error bars represent standard error. Linear, quadratic, and cubic effects had p-values of 0.0580, 0.1206, and 0.1229 in 2003 (A) and 0.5190, 0.1118, and 0.0666 in 2004 (B), respectively.

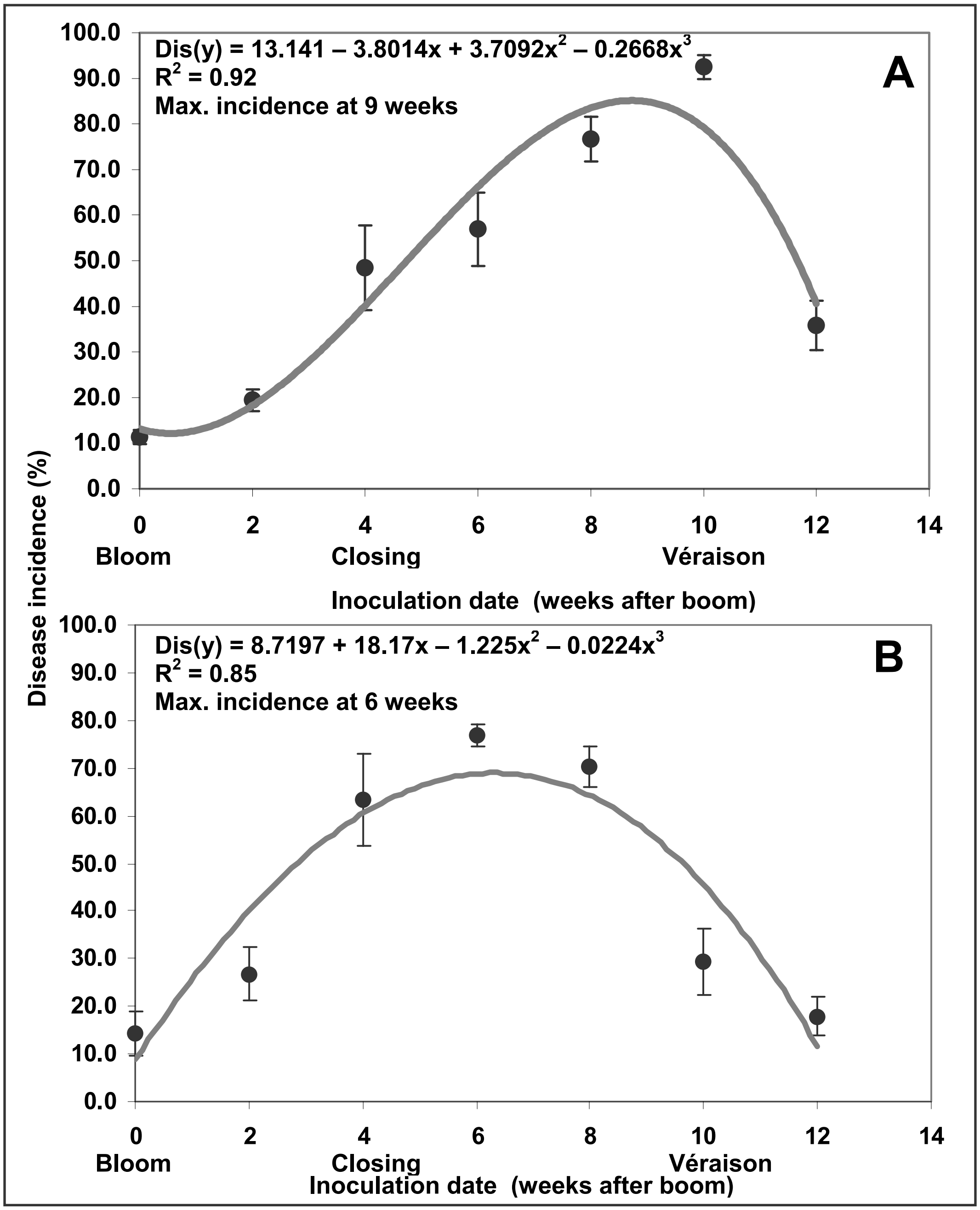

Figure 4. Relationship between disease incidence and inoculation date for Cabernet Franc fruit from bloom until 2 weeks after véraison in 2003 (A) and 2004 (B). Each point represents the mean disease incidence at harvest for a whole vine or cordon (n = 4). Error bars represent standard error. Linear, quadratic, and cubic effects had p-values of 0.6960, 0.1322, and 0.0736 in 2003 (A) and 0.1945, 0.6211, and 0.8658 in 2004 (B), respectively.

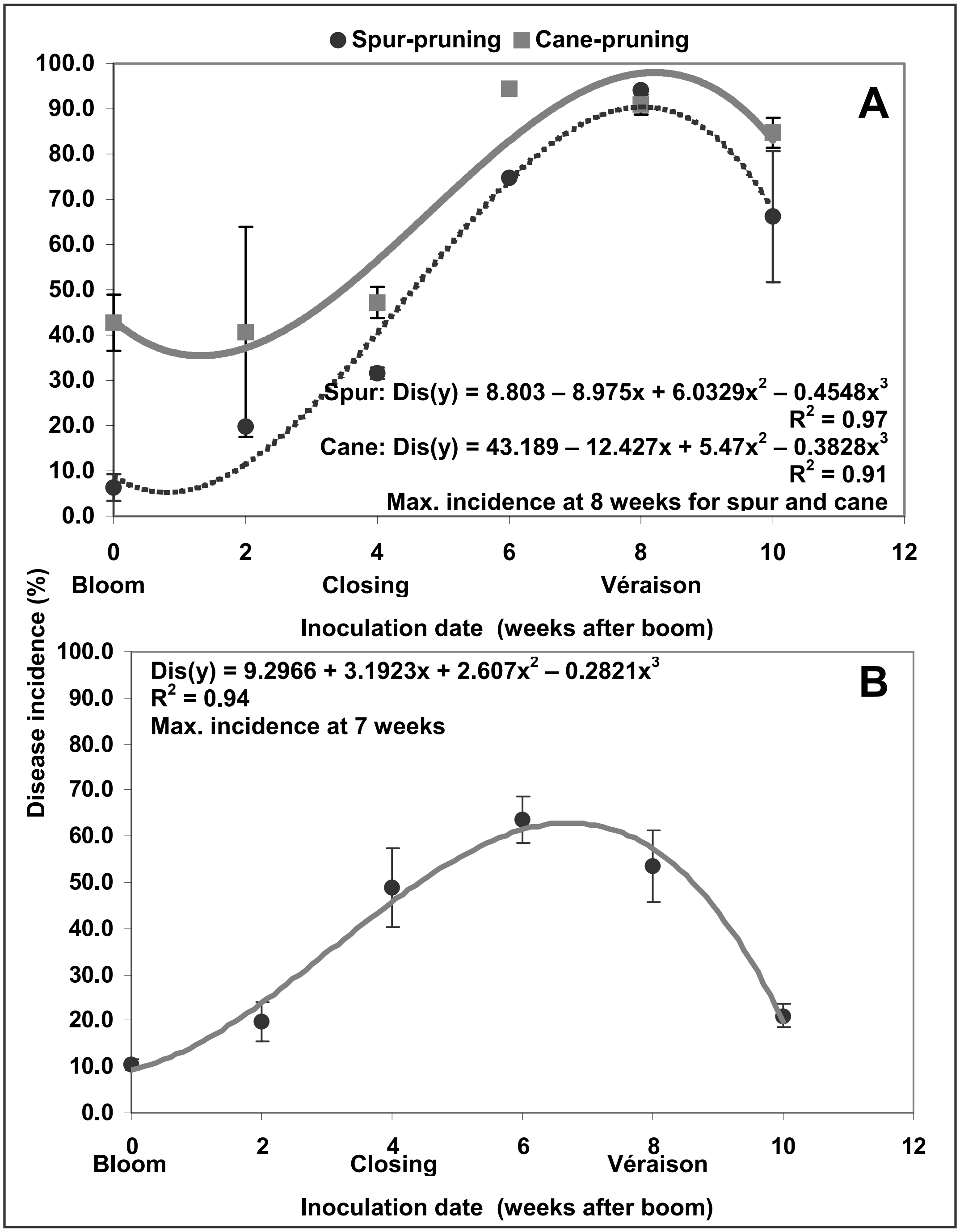

Figure 5. Relationship between disease incidence and inoculation date for Merlot fruit from bloom until 2 weeks after véraison in 2003 (A) and 2004 (B). Each point represents the mean disease incidence at harvest for a whole vine or cordon (n = 4). Error bars represent standard error. Linear, quadratic, and cubic effects had p-values of 0.4171, 0.1107, and 0.0874 for spur-pruning and 0.3871, 0.1915, and 0.1742 for cane-pruning in 2003 (A) and 0.5591, 0.1499, and 0.0639 in 2004 (B), respectively.

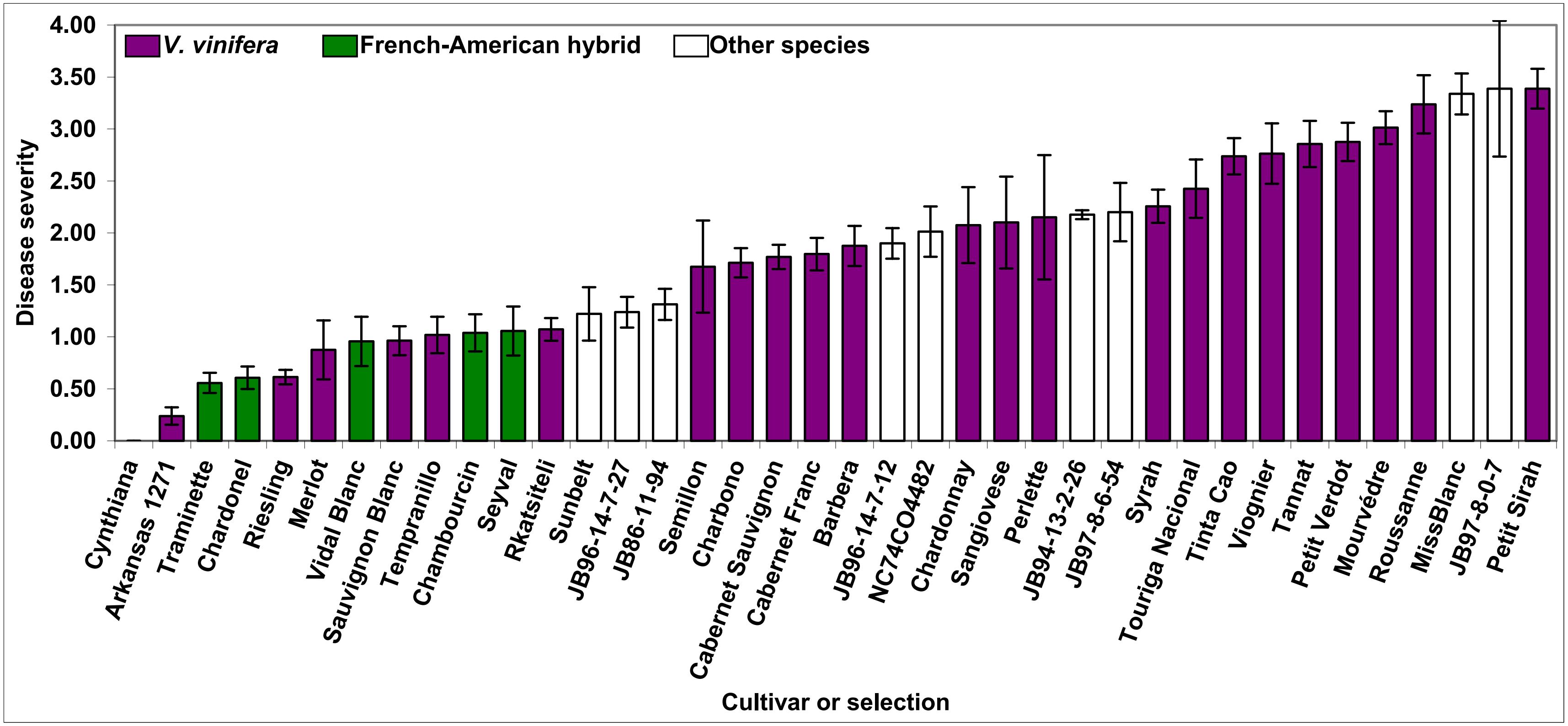

Figure 6. Susceptibility of *Vitis* cultivars and selections tested to *Greeneria uvicola*. Bars represent the mean severity for each cultivar (n = 8) based on inoculations of detached fruit with *G. uvicola* isolate WB5, and error bars represent standard error. Disease severity was evaluated 7 days after inoculation, using the following scale: 0 = no infection, 1 = 1 to 5% fruit surface with visible symptoms, 2 = 6 to 15%, 3 = 16 to 50%, and 4 ≥ 51%. When combined over species, means for *V. vinifera* and "other" species differed significantly from French-American hybrids according to the Waller-Duncan *k*-ratio *t* test where $k = 100$ with MSD = 0.30, $R^2 = 0.29$, and CV = 59.65. *V. aestivalis* 'Cynthiana' with a mean severity of 0.00 and *V. labrusca* 'Sunbelt' were grouped into "other" species.

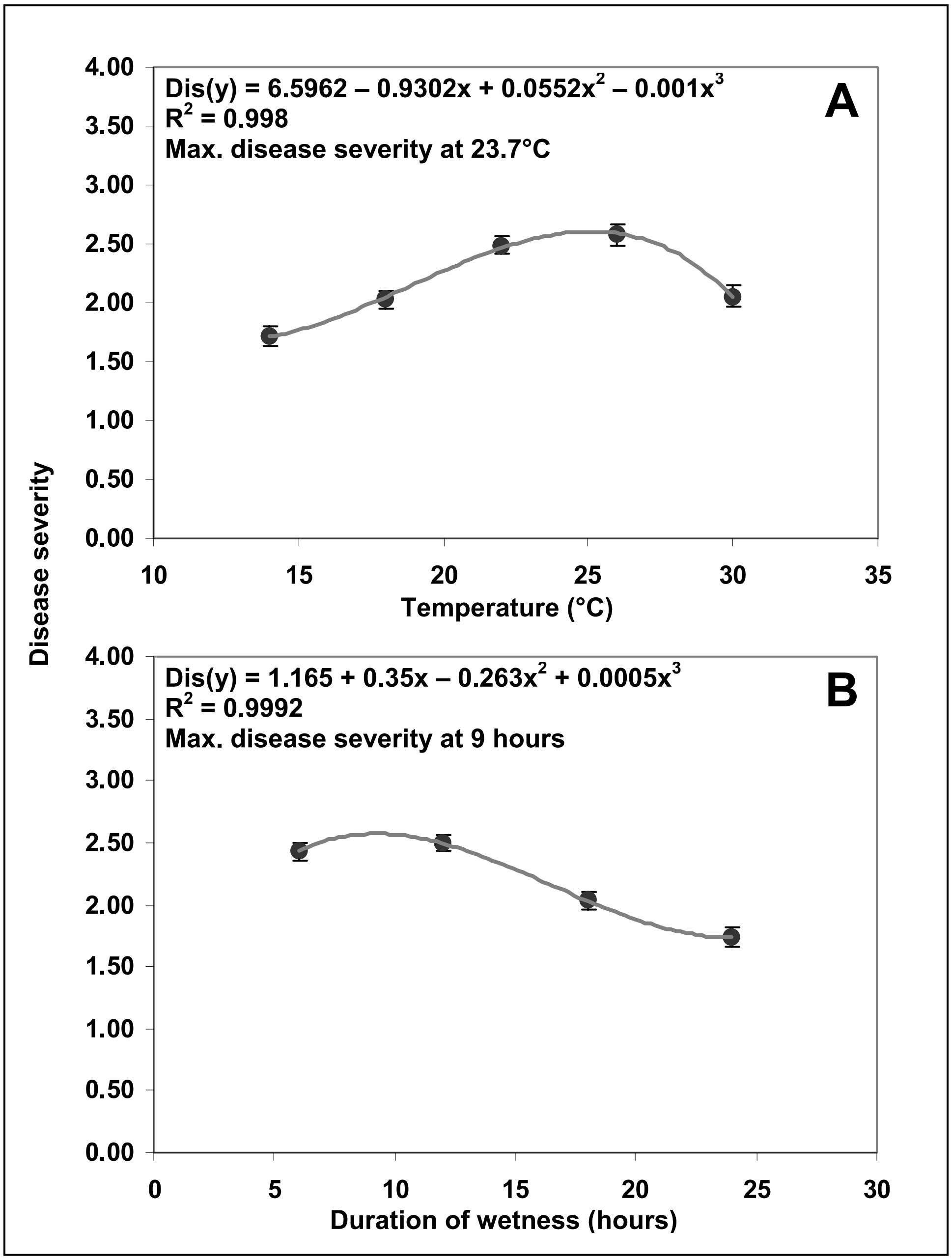

Figure 7. Relationship between disease severity and temperature (A) and duration of wetness (B) on bitter rot infection in *V. vinifera* fruit. Each point represents the mean severity (n = 12 for A, n = 15 for B) for each level based on inoculations of detached fruit with *G. uvicola* isolate WB5, and error bars represent standard error. Disease severity was evaluated 9 to 10 days after inoculation, using the following scale: 0 = no infection, 1 = 1 to 5% fruit surface with visible symptoms, 2 = 6 to 15%, 3 = 16 to 50%, and 4 ≥ 51%. Linear, quadratic, and cubic effects had p-values of 0.1242, 0.0987, and 0.0848 in 2003 (A) and 0.0665, 0.0652, and 0.0729 in 2004 (B), respectively.

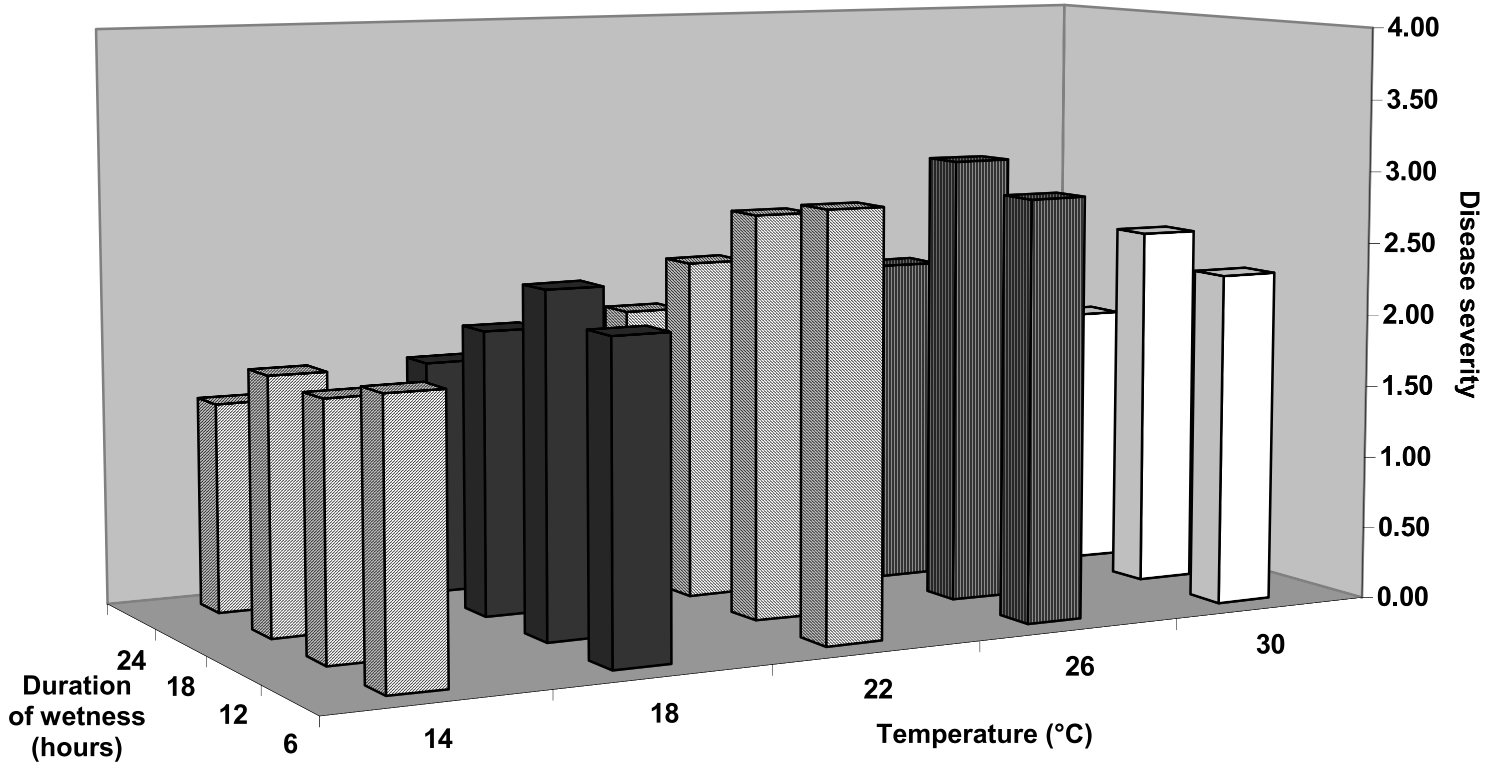

Figure 8. Interaction of temperature and duration of wetness on bitter rot infection in *Vitis vinifera* fruit. Bars represent the mean severity for each temperature and wetness combination based on inoculations of detached fruit with *G. uvicola* isolate WB5. Disease severity was evaluated 9 to 10 days after inoculation, using the following scale: 0 = no infection, 1 = 1 to 5% fruit surface with visible symptoms, 2 = 6 to 15%, 3 = 16 to 50%, and 4 ≥ 51%. For temperature and duration of wetness interaction: F = 4.35 (p = 0.0001).

Appendix 7.1. Susceptibility of popular cultivars grown in North Carolina to bitter rot.

Susceptibility to Bitter Rot[a]	**Cultivar**	**Species**
Resistant	Cynthiana	*V. aestivalis*
Moderately Resistant	Chardonel	French-American hybrid
	Merlot	*V. vinifera*
	Riesling	*V. vinifera*
	Sauvignon Blanc	*V. vinifera*
	Traminette	French-American hybrid
	Vidal Blanc	French-American hybrid
Slightly Susceptible	Cabernet Franc	*V. vinifera*
	Cabernet Sauvignon	*V. vinifera*
	Chambourcin	French-American hybrid
	Seyval	French-American hybrid
	Tempranillo	*V. vinifera*
Moderately Susceptible	Chardonnay	*V. vinifera*
	Sangiovese	*V. vinifera*
	Syrah	*V. vinifera*
	Viognier	*V. vinifera*
Highly Susceptible	Mourvédre	*V. vinifera*
	Petit Verdot	*V. vinifera*

[a] Susceptibility to bitter rot assigned based on severity of inoculations of detached fruit harvested at ≥16°Brix and incubated 26°C under continuous light for 7 days.

Appendix 7.2 Output from SAS PROC GLM for disease severity among isolates from *V. vinifera* and *V. rotundifolia*.

```
                                   The GLM Procedure

Dependent Variable: disease severity

                                            Sum of
      Source                      DF          Squares     Mean Square    F Value    Pr > F

      Model                       11      69.29115909      6.29919628      17.98    <.0001

      Error                       76      26.63083636      0.35040574

      Corrected Total             87      95.92199545


                       R-Square     Coeff Var      Root MSE      disease severity Mean

                       0.722370      38.07314      0.591951      1.554773


      Source                      DF        Type I SS     Mean Square    F Value    Pr > F

      run                          1       0.13291364      0.13291364       0.38    0.5398
      isolate                     10      69.15824545      6.91582455      19.74    <.0001


      Source                      DF      Type III SS     Mean Square    F Value    Pr > F

      run                          1       0.13291364      0.13291364       0.38    0.5398
      isolate                     10      69.15824545      6.91582455      19.74    <.0001


                             Waller-Duncan K-ratio t Test for disease severity
  NOTE: This test minimizes the Bayes risk under additive loss and certain other
assumptions.
                            Kratio                              100
                            Error Degrees of Freedom             76
                            Error Mean Square              0.350406
                            F Value                           19.74
                            Critical Value of t             1.80451
                            Minimum Significant Difference   0.5341
                   Means with the same letter are not significantly different.

                    Waller Grouping          Mean       N    isolate

                                  A        2.9875       8    b
                                  A
                                  A        2.9500       8    c

                                  B        2.1000       8    e
                                  B
                             C    B        1.9875       8    d
                             C    B
                             C    B        1.6500       8    g
                             C    B
                             C    B        1.6000       8    f
                             C
                             C    D        1.4875       8    h
                                  D
                             E    D        1.0600       8    j
                             E
                             E    F        0.5925       8    i
                                  F
                                  F        0.4875       8    k
                                  F
                                  F        0.2000       8    a
```

Appendix 7.3 Output from SAS PROC GLM for disease incidence in inoculation dates of Chardonnay in 2004.

```
                                        The GLM Procedure

Dependent Variable: disease incidence

                                                Sum of
      Source                      DF          Squares     Mean Square    F Value    Pr > F

      Model                       11       0.97323708      0.08847610       8.80    0.0004

      Error                       12       0.12066083      0.01005507

      Corrected Total             23       1.09389791

                       R-Square     Coeff Var      Root MSE      disease incidence Mean

                       0.889696      30.18663      0.100275      0.332183

      Source                      DF        Type I SS     Mean Square    F Value    Pr > F

      inocdate                    5      0.87734673      0.17546935      17.45    <.0001
      rep(inocdate)               6      0.09589035      0.01598173       1.59    0.2327

      Source                      DF      Type III SS     Mean Square    F Value    Pr > F

      inocdate                     5       0.27488851      0.05497770       5.47    0.0075
      rep(inocdate)                6       0.09589035      0.01598173       1.59    0.2327
```

Appendix 7.4 Output from SAS PROC GLM for disease severity in *Vitis* cultivars and selections.

```
                                 The GLM Procedure

Dependent Variable: disease severity

                                             Sum of
      Source                      DF        Squares     Mean Square    F Value    Pr > F

      Model                       35    119.6318119       3.4180518       4.47    <.0001

      Error                      220    168.1135377       0.7641524

      Corrected Total            255    287.7453496

                       R-Square     Coeff Var      Root MSE      disease severity Mean

                       0.415756      50.09614      0.874158      1.744961


      Source                      DF      Type I SS     Mean Square    F Value    Pr > F

      sugar                        8    23.17238326      2.89654791       3.79    0.0003
      color                        1     0.15056025      0.15056025       0.20    0.6576
      ripening                     2    23.46317450     11.73158725      15.35    <.0001
      ripening*sugar               8     8.52280615      1.06535077       1.39    0.2002
      species                      2    32.30406505     16.15203253      21.14    <.0001
      sugar*species                7    16.91043941      2.41577706       3.16    0.0033
      color*species                2     0.25393246      0.12696623       0.17    0.8470
      ripening*species             3    10.93706475      3.64568825       4.77    0.0030
      color*sugar                  2     3.91738605      1.95869302       2.56    0.0794


      Source                      DF    Type III SS     Mean Square    F Value    Pr > F

      sugar                        8    13.94164448      1.74270556       2.28    0.0231
      color                        1    12.78370215     12.78370215      16.73    <.0001
      ripening                     2     3.49037904      1.74518952       2.28    0.1043
      ripening*sugar               5     3.74771245      0.74954249       0.98    0.4303
      species                      2    21.05704790     10.52852395      13.78    <.0001
      sugar*species                7     5.54815592      0.79259370       1.04    0.4059
      color*species                1     1.87005625      1.87005625       2.45    0.1192
      ripening*species             3    10.19139034      3.39713011       4.45    0.0047
      color*sugar                  2     3.91738605      1.95869302       2.56    0.0794


         Tests of Hypotheses Using the Type III MS for ripening*sugar as an Error Term

      Source                      DF    Type III SS     Mean Square    F Value    Pr > F

      sugar                        8    13.94164448      1.74270556       2.33    0.1839
      ripening                     2     3.49037904      1.74518952       2.33    0.1929
      color                        1    12.78370215     12.78370215      17.06    0.0091


         Tests of Hypotheses Using the Type III MS for sugar*species as an Error Term

      Source                      DF    Type III SS     Mean Square    F Value    Pr > F

      species                      2    21.05704790     10.52852395      13.28    0.0041
```

Appendix 7.5 Output from SAS PROC GLM for disease severity in detached *V. vinifera* fruit exposed to different temperature and wetness conditions.

```
                                 The GLM Procedure

Dependent Variable: disease severity

                                        Sum of
      Source                      DF         Squares     Mean Square    F Value    Pr > F

      Model                      179     166.6631733       0.9310792       1.22    0.0565

      Error                      360     274.1096000       0.7614156

      Corrected Total            539     440.7727733


                 R-Square     Coeff Var      Root MSE      disease severity Mean

                 0.378116      40.14580      0.872591      2.173556


      Source                      DF       Type I SS     Mean Square    F Value    Pr > F

      rep                          2      0.70663111      0.35331556       0.46    0.6291
      cv                           2      9.70949778      4.85474889       6.38    0.0019
      rep(cv)                      4      0.54148444      0.13537111       0.18    0.9498
      temp                         4     54.14321037     13.53580259      17.78    <.0001
      cv*temp                      8     10.25084296      1.28135537       1.68    0.1011
      rep*temp(cv)                24      4.89390667      0.20391278       0.27    0.9998
      wet                          3     51.21682074     17.07227358      22.42    <.0001
      cv*wet                       6      3.80979704      0.63496617       0.83    0.5442
      temp*wet                    12      8.77769037      0.73147420       0.96    0.4863
      cv*temp*wet                 24      7.48855852      0.31202327       0.41    0.9946
      rep*temp*wet(cv)            90     15.12473333      0.16805259       0.22    1.0000


      Source                      DF     Type III SS     Mean Square    F Value    Pr > F

      rep                          2      0.70663111      0.35331556       0.46    0.6291
      cv                           2      9.70949778      4.85474889       6.38    0.0019
      rep(cv)                      4      0.54148444      0.13537111       0.18    0.9498
      temp                         4     54.14321037     13.53580259      17.78    <.0001
      cv*temp                      8     10.25084296      1.28135537       1.68    0.1011
      rep*temp(cv)                24      4.89390667      0.20391278       0.27    0.9998
      wet                          3     51.21682074     17.07227358      22.42    <.0001
      cv*wet                       6      3.80979704      0.63496617       0.83    0.5442
      temp*wet                    12      8.77769037      0.73147420       0.96    0.4863
      cv*temp*wet                 24      7.48855852      0.31202327       0.41    0.9946
      rep*temp*wet(cv)            90     15.12473333      0.16805259       0.22    1.0000


            Tests of Hypotheses Using the Type III MS for rep(cv) as an Error Term

      Source                      DF     Type III SS     Mean Square    F Value    Pr > F

      rep                          2      0.70663111      0.35331556       2.61    0.1882
      cv                           2      9.70949778      4.85474889      35.86    0.0028
```

Appendix 7.5 (continued)

```
    Tests of Hypotheses Using the Type III MS for rep*temp(cv) as an Error Term

Source                      DF     Type III SS     Mean Square    F Value    Pr > F

temp                         4     54.14321037     13.53580259      66.38    <.0001
cv*temp                      8     10.25084296      1.28135537       6.28    0.0002

  Tests of Hypotheses Using the Type III MS for rep*temp*wet(cv) as an Error Term

Source                      DF     Type III SS     Mean Square    F Value    Pr > F

wet                          3     51.21682074     17.07227358     101.59    <.0001
cv*wet                       6      3.80979704      0.63496617       3.78    0.0021
temp*wet                    12      8.77769037      0.73147420       4.35    <.0001
cv*temp*wet                 24      7.48855852      0.31202327       1.86    0.0195
```