Tolerance to Pierce's Disease and the Associated Rickettsia-like Bacterium in Muscadine Grape[1]

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Abstract. Rickettsia-like bacteria, implicated as the casual agent of Pierce's disease, were easily observed by electron microscopy in the xylem of muscadine grape (*Vitis rotundifolia* Michx.) with symptoms of Pierce's disease. The symptoms included severe stunt, marginal leaf burn, and dieback. The bacterium was observed only in the tracheary elements of the xylem.

Differences in levels of tolerance to Pierce's disease were found in muscadine cultivars and breeding selections. Muscadine cultivars and selections developed at Experiment, GA were generally more tolerant to the disease than those developed at Meridian, MS or Raleigh, NC, however, there were some highly tolerant cultivars from all 3 locations. Cultivars from MS were much more tolerant to the disease than were selections from that location which were never considered acceptable for release to commercial growers.

Pierce's disease is the principal factor responsible for the failure of both the European-type (*Vitis vinifera* L.) and American-type (*Vitis labrusca* L.) bunch grapes in the southeastern United States (2, 5, 8). Maintenance of a productive lifespan of grapes in this area requires the use of cultivars resistant to this disease. The only grapes known to be resistant, or tolerant, to Pierce's disease are *Vitis* species native to the Gulf Coastal Plain of the United States (2). The grape industry in the southeastern U. S. is based on these resistant *Vitis* species.

Muscadine grape production (*Vitis rotundifolia* Michx.) is a thriving and rapidly expanding industry in the southeastern states. A major factor in the popularity of muscadine grapes in this area is their high degree of tolerance or resistance to Pierce's disease, which is exemplified by their excellent vigor and longevity (5). However, symptoms of Pierce's disease have been observed in muscadine grapes (3, 6). The symptoms were usually mild, but indexing indicated the presence of the disease (6).

In recent studies the causal agent of Pierce's disease, long considered to be a virus, was shown most likely to be a fastidious bacterium resembling a rickettsia (1, 4). Diagnosis of the disease by symptom expression alone is difficult, as symptom expression may be influenced by many environmental factors. Some of the symptoms may also be confused with nutrient deficiencies. Our primary purpose was to determine whether the rickettsia-like bacterium associated with Pierce's disease could be found in muscadine grapes with symptoms typical of the disease. Differences in the incidence of symptoms among muscadine cultivars were also investigated.

Materials and Methods

The muscadine grape planting at the Agricultural Research Center (ARC), Leesburg, contains over 50 cultivars. Sixteen cultivars were planted in 1959, and additional cultivars were planted between 1963 and 1968. Numbered breeding selections were obtained from: 1) US Horticultural Field Station, Meridian, MS; 2) GA Experiment Station, Experiment, GA; and 3) NC Experiment Station, Raleigh, NC.

Samples for electron microscopy were taken from vines having symptoms of Pierce's disease. All vines sampled were studied and some had leaf margin necrosis. Small tissue samples, 0.5 to 1.0 mm long, were taken from petioles and midveins of leaves. The tissue samples were fixed in 2% glutaraldehyde-2% paraformaldehyde in 0.05 M collidine buffer, pH 7.3, for 2 hrs at 4°C, followed by five 10-min rinses in 0.1 M collidine buffer. The samples were then fixed in 1% OsO_4 overnight at 4°C. They were washed in deionized water, dehydrated in an alcohol and acetone series, and embedded in a Spurr epoxy-resin mixture[3] (7). Thin sections were cut on an LKB ultramicrotome[3], stained with uranyl acetate and lead citrate, and examined with a Phillips EM-300 electron microscope[3].

Vines were rated for severity of Pierce's disease symptoms on September 19. Ratings were made on a scale of 0 to 5 with 0 indicating a dead plant, and 5 indicating a symptomless vine.

Results

Muscadine grapes are ordinarily vigorous and long-lived in FL. In recent years, however, symptoms resembling those of Pierce's disease have been observed in muscadine grapes in the vineyard at the ARC, Leesburg. In some vines these symptoms were severe (Fig. 1); whereas, in others they were mild. The symptoms included severe decline of vine vigor, reduced yields, dieback of shoot tips, leaf margin necrosis, and death of the vine. In FL, these symptoms have often been attributed to poor adaptability, cold damage, nutrient deficiency, drought, or some other environmental conditions.

Two 'Pride' vines, 1 'Scuppernong' vine, and 1 'U.S. 19' vine were chosen for electron microscopy because of severe stunt, marginal leaf burn, and dieback symptoms. Bacteria, resembling the rickettsia-like bacterium associated with Pierce's disease of bunch grapes, were readily observed in the xylem of these muscadine grapes (Fig. 2). The bacteria were commonly 0.35-0.50 μm by 1.0-3.0 μm rod-shaped bodies with well-defined cell walls. The cell walls were usually rippled as were the walls of the rickettsia-like bacterium associated with the disease in bunch grapes (1, 4). The bacteria were observed only in the tracheary elements of the xylem of the muscadines and not in either the xylem parenchyma or the phloem.

The rickettsia-like bacteria were often surrounded by a transparent or slightly granular substance (Fig. 2), as they were in bunch grapes. This granular matrix was observed in many mounts to be much more electron-dense in muscadine than in bunch grapes (Fig. 3). The lumen of some xylem vessels contained such a dense, dark matrix that bacteria could not be seen within the matrix.

Preliminary observations indicated that incidence and severity of symptoms of Pierce's disease varied among muscadine cultivars and breeding selections; therefore, all vines which had been in the ARC, Leesburg, vineyard for 3 years or

[1]Received for publication February 16, 1974. FL Agr. Exp. Sta. J. Series No. 5315.

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[3]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

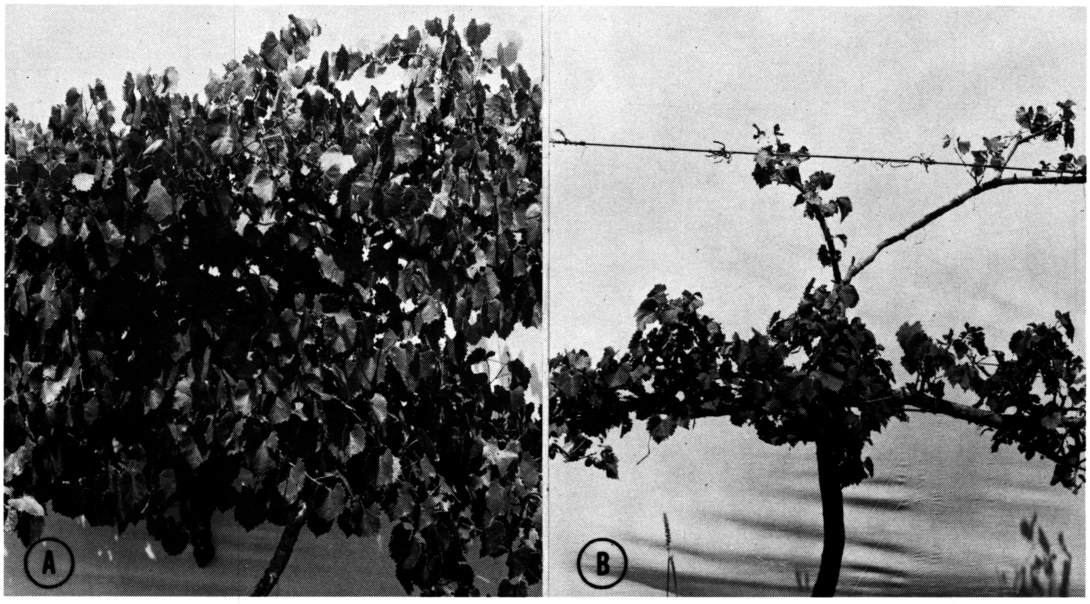

Fig. 1. Pierce's disease symptoms in muscadine grapes. (A) Healthy, vigorous vine, and (B) infected vine with poor vigor and severe dieback symptoms.

more were rated for symptoms (Table 1). Since there were only a few vines of each cultivar in the test, the incidence and ratings given here should not be interpreted as representing the absolute level of tolerance of the cultivars to Pierce's disease. However, it is obvious that cultivars such as 'Lucida', 'Scuppernong', 'Pride', and 'Roanoke' were less tolerant of the disease than 'Higgins', 'Hunt', 'Chief', and 'Southland', which have been in the vineyard for 14 years and are still free of symptoms.

When breeding selections and cultivars are grouped by place of origin, large differences in mean tolerance to Pierce's disease are observed (Table 2). Muscadines developed at the GA Experiment Station appeared much more tolerant than those from NC or MS. The apparent difference in tolerance between selections and cultivars from NC and those from MS may be real, but it may also be at least partially explained by the difference in mean age of the vines, or years of exposure to the disease. The incidence of Pierce's disease is also much less in cultivars from MS than in breeding selections which were not released. In NC muscadines, and to some extent those from GA, the situation is the reverse of that in MS in that the cultivars have approximately 3 times as much Pierce's disease as the

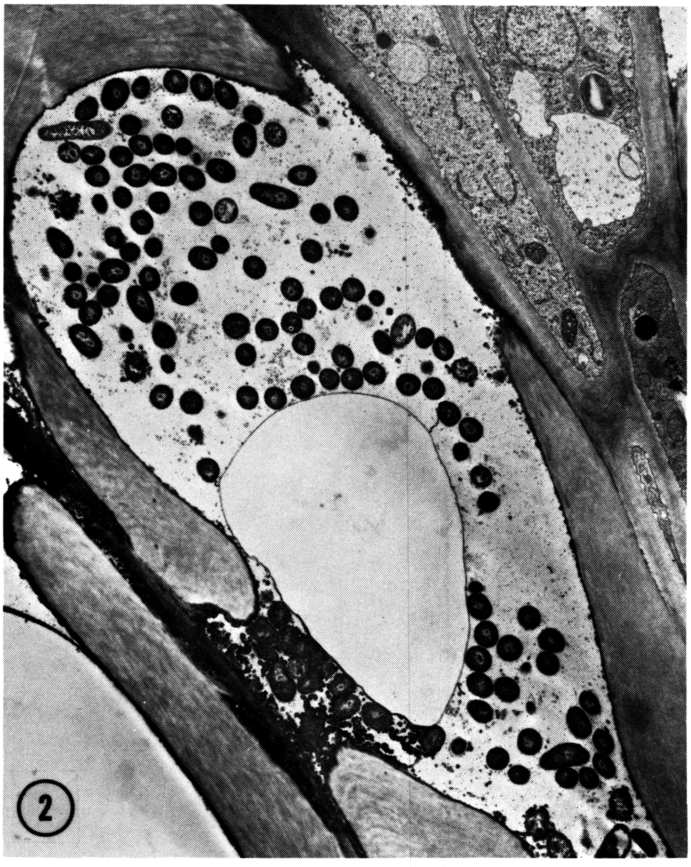

Fig. 2. Transverse section through a xylem vessel in a leaf vein showing a common distribution pattern of rickettsia-like bacteria. X 6,200.

Table 1. Incidence and severity of Pierce's disease symptoms on various muscadine grape cultivars.

Cultivar	Vine age[z] (yrs)	Incidence of symptoms[y]	Dead vines (no.)	Mean disease rating[x]
Higgins	9-14	0/7	0	5.0
Fry	7-8	0/4	0	5.0
Hung	14	0/2	0	5.0
Chief	14	0/3	0	5.0
Southland	14	0/3	0	5.0
Magnolia	6-10	0/3	0	5.0
Creek	14	0/1	0	5.0
Carlos	5	0/2	0	5.0
Jumbo	3-6	0/4	0	5.0
Coward	3-8	0/4	0	5.0
Albermarle	5-10	1/3	0	4.7
Dearing	14	1/3	0	4.7
Magoon	8-14	1/6	0	4.3
Topsail	14	1/1	0	4.0
Pamlico	6	1/2	0	3.5
Bountiful	10	1/3	1	3.3
Roanoke	6-10	2/3	1	3.0
Pride	5-6	3/3	0	2.7
Scuppernong	6-14	3/3	1	1.0
Lucida	14	1/1	1	0

[z]Numbers represent total years that vines have been in vineyard. More than 1 planting of some cultivars were made and the age range of vines of these cultivars is given.

[y]Number of vines with Pierce's disease symptoms over total nuber of vines.

[x]Individual vines rated on a scale of 0 to 5 with 0, dead; 3, moderate Pierce's disease symptoms; and 5, healthy.

Fig. 3. Transverse section through the xylem of a leaf vein showing massive accumulation of dense granular material surrounding the bacteria and filling the xylem vessel. W = cell wall. X 7,500.

nonreleased selections. Total loss, or death, of the muscadine vines has been low in all groups except the nonreleased breeding selections from MS in which nearly 40% of the vines have been lost.

Discussion

Two groups of researchers have recently shown a rickettsia-like bacterium apparently to be causally associated with Pierce's disease of grape, although neither group fulfilled Koch's postulates (1, 4). The occurrence of rickettsia-like bacteria in muscadine grapes with symptoms resembling those of the disease supports the hypothesis that muscadines are susceptible to Pierce's disease. However, they are much more tolerant of the disease than are European or American-type bunch grapes. This is proven by their vigor and longevity in the southeastern US where the disease is endemic and prohibits a successful European or American-type bunch grape industry.

Our results confirm the finding of Loomis (6) in MS that muscadine grapes are not immune to Pierce's disease. That report stated, however, that "all muscadine grapes are long-lived. Disease symptoms in them were slight and generally inconclusive." This was often the case at the ARC, Leesburg, however, we have observed severe, typical symptoms and even death of young vines. Perhaps, this difference in degree of symptoms in these 2 reports is due to environmental differences between the locations, which result in greater disease pressure on vines at Leesburg.

Different levels of tolerance to Pierce's disease were obvious in muscadine cultivars growing in the Leesburg vineyard. Although half of the cultivars were symptomless, this certainly does not mean that they are immune to the disease. More likely, they simply have a higher level of tolerance and are able to keep the causal agent at sub-clinical populations. Five or 6 cultivars appeared to have intermediate levels of tolerance, but tests involving more vines would be required to rank their tolerances. At lease 4 cultivars ('Lucida', 'Scuppernong', 'Pride', and 'Roanoke') appeared to have very low tolerance.

Table 2. Relationship of the incidence of Pierce's disease symptoms to the origin of the muscadine cultivars and breeding selections.

Origin	Mean age of vines (yrs)	Cultivars or selections with symptoms (%)[z]	Vines with symptom (%)[y]	Dead vines (%)
Combined cultivars and selections				
Georgia	8.3	18.1 (22)	12.0 (50)	2.0
North Carolina	5.9	36.0 (25)	24.5 (53)	7.5
Mississippi	12.5	72.7 (11)	41.7 (36)	22.2
Breeding selections				
Georgia	7.4	11.1 (9)	5.6 (18)	0
North Carolina	5.9	22.2 (18)	13.9 (36)	5.6
Mississippi	12.8	83.3 (6)	66.7 (18)	38.9
Cultivars				
Georgia	8.9	23.1 (13)	15.6 (32)	3.1
Mississippi	12.3	60.0 (5)	16.7 (18)	5.6
North Carolina	7.4	71.4 (7)	47.1 (17)	11.8

[z]Percentage of cultivars or selections in which at least 1 vine has Pierce's disease symptoms. The number in parenthesis is the total number of cultivars or selections.

[y]Percentage of total vines which have Pierce's disease symptoms. The number in parenthesis is the total number of vines.

The observed differences in the mean tolerance to Pierce's disease in the muscadines developed at 3 locations in the southeastern US was unexpected. The higher susceptibility of muscadines developed in Raleigh, NC may be explained by the fact that Raleigh is almost out of the northern edge of the area where the disease is endemic. This could mean that there is not enough selection pressure for Pierce's disease tolerance at Raleigh to eliminate the susceptible plants from their breeding program. These cultivars may then develop symptoms when they are grown in an area where the disease is more severe, such as FL. Other factors involved in the differences may include: 1) differences in levels of tolerance in original muscadine selections on which the breeding programs at the various locations were based; 2) differences in goals of the various programs, such as high yield, large fruit, etc.; and 3) differences in amount of inbreeding.

The large difference in tolerance of MS breeding selections and cultivars may indicate that tolerance to Pierce's disease was a major factor in determining performance excellence of a muscadine in their tests for release acceptibility. In selecting muscadines in the southeastern U.S. for vigor, yield, and adaptability one may primarily be selecting for Pierce's disease tolerance.

In summary, it is obvious that there is susceptibility to Pierce's disease in muscadines and that differences in tolerance do exist among muscadine cultivars. Highly tolerant muscadine cultivars are long lived, vigorous, and quite productive in the southeastern US where the disease is endemic. Muscadine grape breeders in this area should select carefully for high levels of tolerance to this disease.

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The Metabolism of Abscisic Acid to a Water Soluble Complex in Apple[1]

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Abstract. When apple (*Malus sylvestris* Mill.) seedlings are fed abscisic acid (ABA), a water soluble complex of ABA and glucose is rapidly formed. This may be a mechanism for inactivating ABA. Similarly, the *trans* isomer of ABA (*trans*-ABA) gives rise to a water soluble complex, which is also presumed to be a glucose complex. Apple seedlings did not convert ABA to *trans*-ABA during feeding experiments, suggesting that small amounts of *trans*-ABA occasionally found in apple seedling extracts may be artifacts.

Living organisms have various mechanisms for metabolizing excessive amounts of many chemicals which they synthesize or absorb. Some substances are complexed with glucose or other simple sugars, resulting in inactivation of the offending molecules. The naturally occurring growth inhibitor, abscisic acid, appears to be inactivated in this manner, at least in certain plants. Milborrow (2) reported that radioactive ABA fed to tomato shoots was rapidly converted to its glucose ester. This substance had earlier been identified from yellow lupin by Koshimizu et al. (1), and was reported to be inactive with regard to its growth inhibiting properties.

Seeley[4] demonstrated the existence of a water soluble complex in apple shoots and buds which could be hydrolyzed to ABA (hyd-ABA). Powell and Seeley (5) briefly described feeding experiments in which ABA and its *trans*-isomer were rapidly converted to hydrolyzable water soluble complexes in apple. This report describes this work in greater detail, and tentatively identifies hyd-ABA as an ABA-glucose complex.

Materials and Methods

ABA from 3 sources was employed. ABA from the Reynolds Tobacco Co. consisted of 4 isomers in equal amounts: (+)-ABA, (-)-ABA, (+)-*trans* ABA, (-)-*trans* ABA. ABA from Shell Research Ltd. and from Hoffmann-La Roche, Inc. consisted of equal amounts of 2 isomers: (+)-ABA and (-)-ABA. The naturally occurring, active substance is believed to be (+)-ABA in most plants.

ABA was injected into apple seedlings by use of a wick of absorbant cotton thread dipped into a small vial of the ABA solution (Fig. 1). Typically 100 μg of Reynolds' or Shell's ABA in 0.1 ml methyl alcohol (MeOH) was added to 0.4 ml water and placed in the vial. All of the liquid disappeared from the vial within a few hours. Some of the liquid was taken up in the transpiration stream of the seedling, but some doubtless evaporated directly into the air. No attempt was made to determine the actual amount of ABA entering the plant. The results presented cannot be regarded as quantitative.

Conversion of ABA and trans-ABA to hydrolyzable forms. Vigorously growing seedlings of 'Northern Spy' apple averaging 45 cm in height were injected just above the first full-sized leaf with 100 μg of Reynolds' ABA (25 μg of each isomer). Controls were injected with only the carrier solvent. Samples were collected on day 0, just before injection, and on days 1, 2, 4, and 14, using 5 seedlings per treatment. Changes in ABA and hyd-ABA, and in *trans*-ABA and hyd-*trans*-ABA were determined. Each of the 45 shoot tips were analyzed separately.

Shoot tips, excised above the point of injection (Fig. 1) were weighed rapidly, dropped into cold MeOH, and extracted by soaking for three 24 hr periods at 2-3°C in the dark, using freshly redistilled MeOH for each period. Extracts were bulked, evaporated to the aqueous phase and fractionated (Fig. 2).

Fraction A (acid fraction) was analyzed for free ABA and *trans*-ABA by a previously described method (7), using GLC columns packed with either 3% DC-200 or 1% XE 60. Fraction B was analyzed for hyd-ABA and hyd-*trans*-ABA in the same manner. The optical isomers were not separated.

Attempted conversion of ABA to trans-ABA. In hundreds of analyses for endogenous ABA in apple tissues, we have seldom detected the *trans*-isomer. Those traces occasionally seen could be either naturally occurring or artifacts (2). It was of interest to determine if apple seedlings can convert ABA to *trans*-ABA when fed physiologically-large amounts.

Twelve vigorously growing 'Northern Spy' apple seedlings were injected with 100 μg of Shell's ABA (which contained none of the *trans*-isomers). Control trees were injected with carrier solvent only. Seedling shoot tips were sampled at days 0, 1, 2, 4 and 14 as in the previous experiment, but 3 trees instead of 5 were used in each treatment. Shoot tips were processed as previously described and analyzed for *trans*-ABA and hyd-*trans*-ABA as well as for free and hyd-ABA. Each shoot tip was analyzed separately.

Identification of hyd-ABA. Five apple seedlings were injected with 100 μg of Hoffmann-La Roche ABA and 4 days later extracted with 30% aqueous MeOH. The hyd-ABA fraction (Fig. 2) was characterized by Milborrow's procedure (2). The pH was adjusted to 8.0, and the hyd-ABA adsorbed on activated charcoal during 18 hrs stirring. The charcoal was eluted with increasing concns of acetone in water, the purest and largest hyd-ABA fraction being eluted with 70% acetone. A brown gum remained after solvent removal. This was dissolved in a few drops of water, and additional impurities were precipitated when 2 mls of MeOH were added. Hyd-ABA in the supernatant fluid was purified to give a single UV-absorbing spot on Silica Gel F-254 TLC plates (Brinkman), using 1-propanol:ethyl acetate:water (3:2:1), and chloroform:MeOH:water (75:22:3). Rf values were 0.90 and 0.40 respectively. Compounds were traced in the various chromatography and separation steps by

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