Vitis caribaea as a source of resistance to Pierce's disease in breeding grapes for the tropics

L. G. Jiménez A. and A. Ingalls

P. O. Box 10135, San José 1000, Costa Rica

Summary: A native Costarican vine, *Vitis caribaea*, was found growing unaffected by Pierce's disease (PD; *Xylella fastidiosa*) in the forests surrounding a dying *V. vinifera* plantation. *V. caribaea* was tested by inoculation, isolation, ELISA and DNA hybridization and in all cases no bacteria were detected. It was decided that *V. caribaea* or Agrá (its Indian name) is resistant or at least highly tolerant to PD. Crosses of *V. vinifera* and *V. caribaea* were made and no compatibility barriers were found, germination of the hybrids seeds was high and a high percentage of fertile plants were produced. Many hybrids were made and planted in the field to test them for resistance to PD.

Since some of the F_1 hybrids do transmit resistance when backcrossed to *V. vinifera*, resistance must be determined by dominant genes. Some F_1 hybrids, although apparently resistant themselves, are either not transmitting resistance or are doing so in a reduced proportion. Several hybrids developed at the University of Florida were tested, one of these, F 5-8, has led to the establishment of the first successful vineyard in Costa Rica.

Key words: Pierce's disease, bacterium, Vitis, resistance, breeding, genetics, tropics, Costa Rica, America.

Introduction

The Spanish have a very old viticultural tradition and it is remarkable that this tradition should have been today lost when they colonized tropical America. It is said that the Spanish government forbade the planting of grapes in their colonies to prevent competition with their own domestic wine production. Undoubtedly in the long history of these colonies thousands of Spanish settlers have tried to establish their own vineyards. Indeed, in our own lifetimes we have known of numerous cases of people who, having planted a vineyard, had some success at first only to be disillusioned a few years later when all the plants died. A similar experience in southern California was described by Pierce (1892).

The symptoms of Pierce's disease (PD) are very similar to those of acute water stress: the edges of the leaves burn and later drop off, the berries shrivel up, and the plant dies. We can distinguish the symptoms of PD from simple drying if we notice that the leaves burn asymmetrically in sections, more on one side than on the other side of the leaf, and that a yellow or red band appears on the border between vital and necrotic sections. The plant seems to recover between attacks leaving a narrow dark line between each successive burn. Eventually the leaves fall from the petioles which stay connected to the stem, the wood of the vine matures unevenly leaving islands of immature wood on mature canes which are then subject to winter damage.

For one hundred years all attempts to discover the cause of this disease were unsuccessful, although much field work was done to establish its wild hosts (grasses and many weeds) (Freitag 1951) and its vectors (leafhoppers) (Hewitt *et al.* 1942). Recently, the possibility of applying modern laboratory techniques and the discovery that tetracycline antibiotics (Hopkins and Mortensen 1971) could suppress symptoms in PD diseased vines suggesting that the disease might be caused by a mycoplasma-like organism, revitalized interest in the disease. Using the electron microscope, rickettsia-like bacteria (RLB) were found to be associated with the disease (Goheen *et al.* 1973; Hopkins 1973). A few years later the causal bacterium was isolated and cultivated (Davis *et al.* 1978). This opened up the possibility of using techniques such as inoculation, ELISA (Raju *et al.* 1980, 1981) and DNA hybridization (Jiménez and Davis 1987).

In 1988 the name *Xylella fastidiosa* was proposed (WELLS *et al.* 1987) for this bacterium. Studies of host-vector-pathogen relationships have shown that PD is endemic in California, the Gulf States (HEWITT 1958), Mexico (RAJU *et al.* 1980), Central America (GOHEEN *et al.* 1979; JIMÉNEZ 1980, 1982) and Venezuela (JIMÉNEZ 1985) and it is of ancient origin.

Preliminary work

Following the discovery (GOHEEN *et al.* 1979) and confirmation (HOPKINS, unpublished; JIMÉNEZ 1980) of PD in Costa Rica, a search was made for material having resistance to this disease. Several hybrids developed by MORTENSEN in Florida were tested, one of these, F 5-8, which was never released in Florida because of its susceptibility to fungus diseases, has led to the establishment of the first successful commercial vineyard in Costa Rica. A native Costarican vine, *Vitis caribaea*, was found growing unaffected by any disease in the forests surrounding a dying *V. vinifera* plantation in Montezuma on the Pacific coast of Costa Rica. It was tested by inoculation, isolation, and ELISA and in all cases either no bacteria were found or they were so low in number that the results were untrustworthy. It was decided that *V. caribaea* or Agrá (its Indian name) is resistant or at least highly tolerant to PD and that it might be a good source of resistance if it could be crossed with *V. vinifera*. In mid forties JOSEPH FENNELL had used *V. caribaea* in some of his crosses at the Inter-American Institute of Agricultural Science in Turrialba, but when this organization was reorganized all his genetic material (FENNEL 1948) was lost to Costa Rica. Turrialba is in a wet tropical rainforest, a notoriously bad climate to grow grapes in.

Starting in 1978, new crosses were made, this time in Montezuma which is in a hot dry tropical forest just on the edge of the climatic zone of maximum dryness in which *V. caribaea* can still be found growing naturally. In some crosses female *V. caribaea* was pollinated with *V. vinifera*, in others *V. vinifera* was hand emasculated and pollinated with *V. caribaea*. No compatibility barriers were found. Germination of the hybrid seed was high and a high percentage of fertile plants were produced. Many F_1 hybrids and backcrosses to *V. vinifera* were made and planted in the field at a distance of 40 cm x 200 cm to test for resistance to PD. The field was prepared for planting by machetti in order to preserve the roots of the native hosts of PD intact. The weeds were allowed to grow freely to facilitate infection of the new plants. Weeds were chopped and insecticides were used only when absolutely necessary. We slowly began to realize that we were confronted with a happy but frustrating circumstance: Whereas in a temperate climate the spread of the disease is more or less slow, once it enters the vine, the latter succumbs in a reasonably short time (3 months to 1 year). In the tropics, however, the spread is very rapid, in 6 months over 80 % of a new planting in Montezuma was contaminated (JIMÉNEZ 1982), and yet after infection the disease develops very slowly within the plants which degenerate over a period of 2-9 years. Cardinal produced high fruit for 3 years despite the fact that typical symptoms of PD were observed throughout this time and bacteria were consistantly isolated from them. In laboratory tests, Costarican strains of PD were found to have virulence similar to those of the North American strains (GOHEEN *et al.* 1979). It is possible that a small difference in virulence not observable in the lab could correspond to a large difference in the field, but it is obviously indesirable and illegal to test this in the open. Instead, HOPKINS (private conversation) attributes this greater longevity to an increased tolerance of plants grown in the tropics: Using good cultural practices, in a tropical climate the plant can sustain constant vigorous growth under conditions of little stress, this allows it to outgrow the damage (to the xylem) caused by the bacteria almost as fast as it is produced. In fact in some microclimates (Alajuela, Costa Rica and Maracaibo, Venezuela) (JIMÉNEZ) *vinifera* grapes can be grown successfully in spite of PD. The frustrating aspect is that we can never be quite sure whether a hybrid we have made is really tolerant or whether waiting just one more year it will die. After waiting so many years to selecting a vine for resistance, it becomes rather uncertain whether it died of PD, another disease or accident.

Materials and methods

It would be very useful to know which percentage of a certain cross can be expected to survive PD in Costa Rica. In Florida, it was shown (MORTENSEN 1968) that using a model of three independent dominant genes where all three are necessary for resistance, one could predict the resistant percentage. Could we expect that Agrá has the same type of resistance mechanism as the wild grapes native to Florida that were used by MORTENSEN? Since there is no significant geographic barrier between Florida and Central America and all of this is inhabited by various species of wild grape which are probably related to each other and all are under the pressure of PD, they might share a common type of resistance. Until we had more information, we went on the hypothesis that this is so. This allowed us to use the backcross system of breeding as long as we were sure we had selected out plants that were not resistant at each generation. But this is exactly what we could not do. In our backcross population, now 8 years old, only 27 % of all seedlings have died, instead of the 88 % predicted by MORTENSEN. Many of the survivors however are loosing vigor and becoming unproductive. Fruit production is the principle stress that plants have to undergo and those that are not under stress can survive better an attack of PD. In order to select effectively, all the plants should be under the same stress. This can be achieved to some extent by considering only the plants that are capable of producing fruit:

$$\frac{\text{Plants that are still fruiting}}{\text{All the plants that would be capable of fruiting in absence of PD}}$$

Here we have entered an unmeasurable term in the denominator which is equal to:

All the plants that have produced at least once in their lifetime + the plants that could have produced but died of PD before they had time to

From previous experience with a quasi randomly selected collection of *V. vinifera* varieties donated by Dr. GOHEEN we found that less than 25 % of *vinifera* seedlings die before they can produce fruit. But about $^1/_2$ of the plants in the denominator are resistant to PD, so we have to add $^1/_2$ of $^1/_4$ or $^1/_8$ to the denominator. This correction is not large and will not be made in the following work, however at any time we can include it by multiplying the result by $^8/_9$.

Now we arrived at our measurable ratio which we will call sustainability:

$$\frac{\text{Number of plants in production}}{\text{Number of plants that have produced at some time } (\times\ ^9/_8)}$$

In doing this we have largely corrected an error in the % survivors due to the fact that some plants die of other causes: extreme susceptibility to fungus, genetic weakness, and accidents. Since most of these affect the plants early in their lives they will be prevented from ever producing fruit and so these plants will not enter into the ratio. The numerator and the denominator are not fixed numbers but variables which depend upon the year of observation:

$$\frac{\text{Number in production [Year]}}{\text{Number that have produced [Based year]}} = \text{Sustainability}$$

The based year is the year we look back at our data and calculated the number of plants that have produced at some time. If we do this too early, our ratio is too high and is close to 1 because exactly the same plants appear in the numerator and denominator. If the base year is adequate, the ratio will be a function of the year observed. To illustrate this we use our oldest backcross population (8 years old) as an example. There are too few plants in each cross to get reasonable curves by cross, but by batching one row of 100 plants of mixed backcrosses we get the following data:

Year	6th	7th	8th
Dead plants	12	15	27
Degenerating	18	39	30
Producing	33	18	16
Have produced	38	38	38
ELISA*			3
% Survivors	88 %	85 %	73 %
Healthy	70 %	46 %	43 %
Sustainability	87 %	47 %	42 %

* The 3 plants showing positive readings were already degenerating.

Among other things this data serves to clear up any doubts about our use of sustainability instead of % healthy plants, here they turn out almost equal. Also it is clear that, no matter how we look at the data, we get a ratio of survivors much higher than that of MORTENSON in Florida.

Results

Fig. 1: Here we present a series of graphs showing the progression of selection for PD against time for individual backcross families.

Fig. 2: At about 3-4 years most plants have started producing fruit, but PD has not yet seriously affected the production and so the curve has its maximum at this time. After this, the curve descends as PD kills and degenerates vines, as we hoped after 6 or 7 years the curve levels off as only plants tolerant to PD will be still producing. Unfortunately this leveling off has not occurred for all crosses.

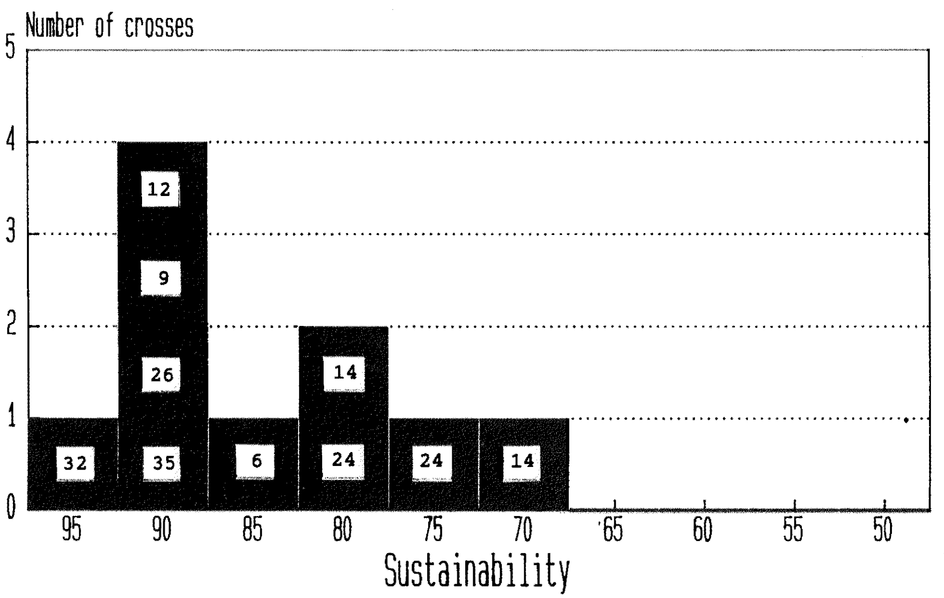

Fig. 1: Sustainability of F_1 hybrids Agrá used as resistant parent. Data from 9-year old plants used.

Fig. 3: It is too soon to tell if this curve will level off leaving a few residual plants or else drop to zero, in which case the 'resistant' parent would not have been transmitting resistance.

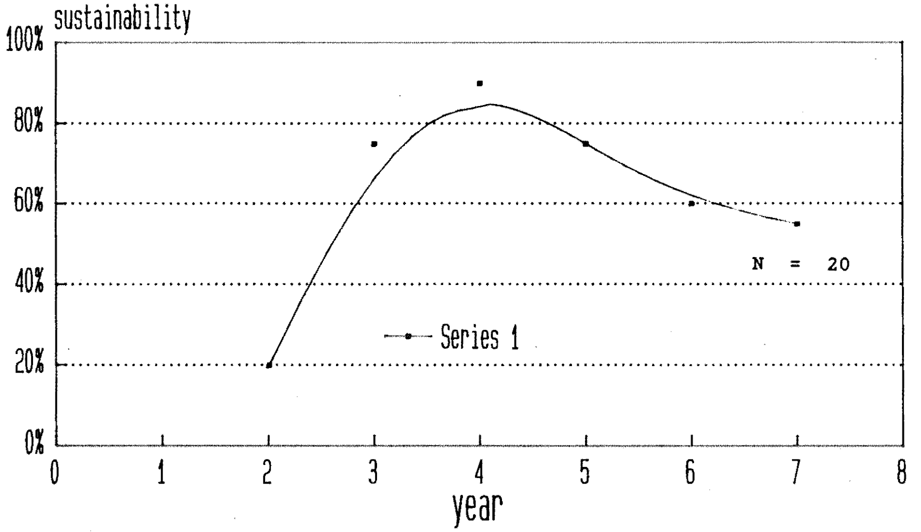

Fig. 2: Sustainability of the progeny of 75 C (32 A 10 x M. Hamburg).

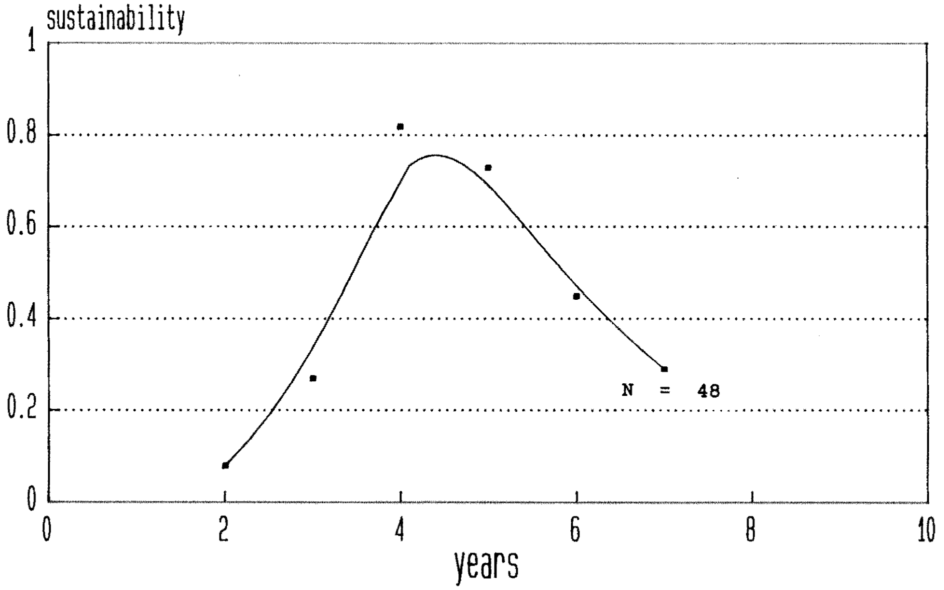

Fig. 3: Sustainability of 73 A (3 C 15 x Ruby Cabernet; 3 C 15 = Petit Sirah x Agrá).

Fig. 4: Plotting together every backcross family with over 15 plants for the denominator (to reduce sampling error) planted in 1982 we notice that the crosses can be divided into two groups, those that seem to be heading for a ratio around 50 % and those that are descending to a much lower level.

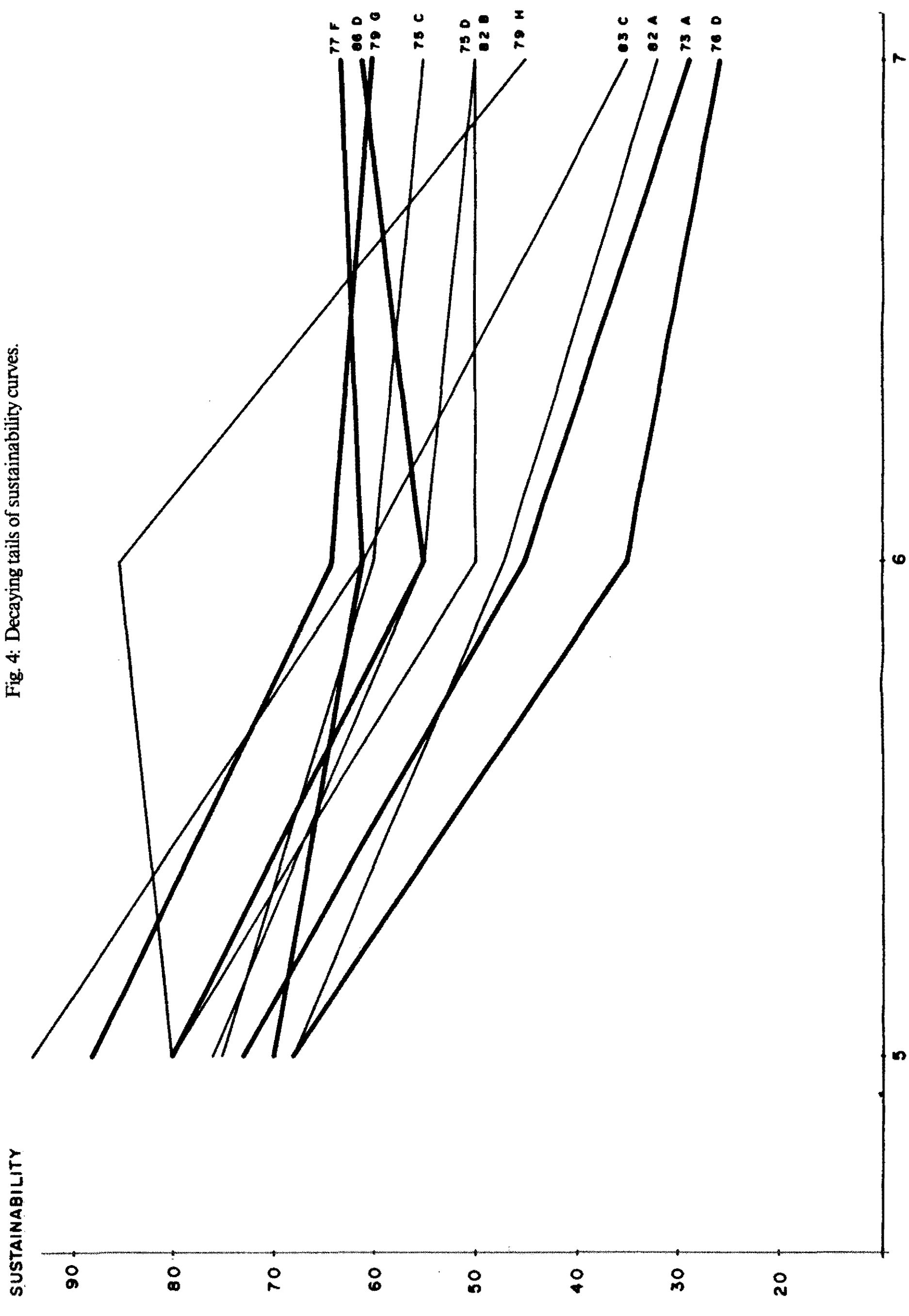

Fig. 4: Decaying tails of sustainability curves.

Fig. 5: Here is set of curves that show 3 different backcrosses all with the same 'resistant' parent 56 C 1. Although 56 C 1 (Aleatico x Agrá) is 9 years old, is still vigorous and has a negative

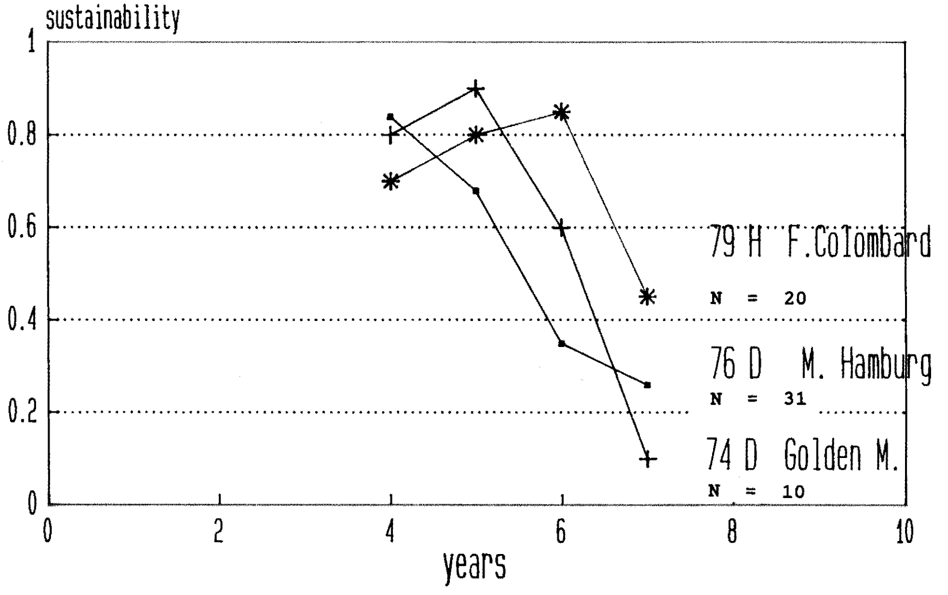

Fig. 5: Sustainability of 3 crosses using 56 C 1 (Agrá x Aleatico).

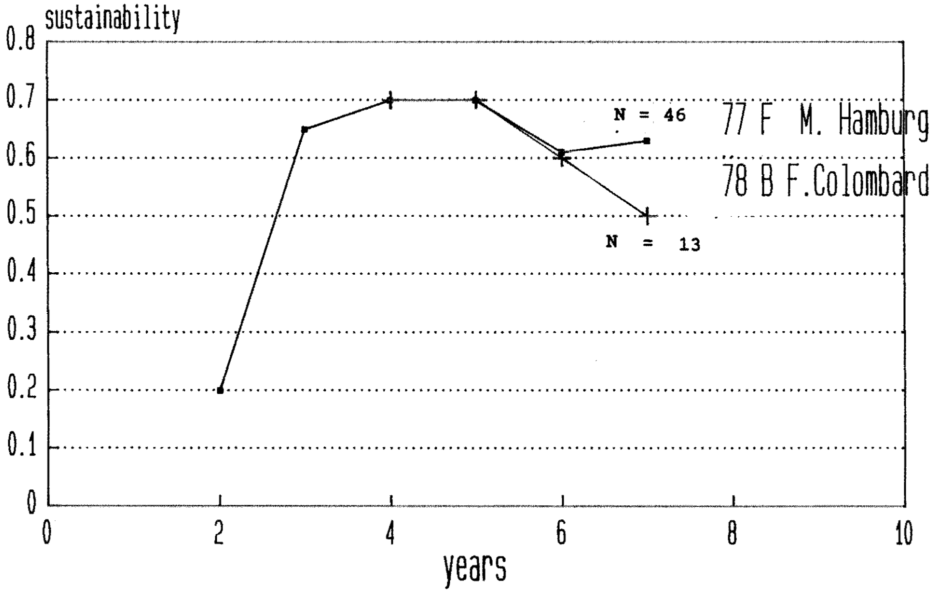

Fig. 6: Sustainability of 2 crosses using 56 C 11 (Agrá x Aleatico).

reaction with ELISA, it does not seem to be transmitting resistance to its progeny. Its productivity has been declining, however, and this seems to be the key to the early spotting of plants low in tolerance to PD. Many of the other plants that are not transmitting full resistance are, however, sustaining their productivity and there is no visible way to distinguish them.

Fig. 6: This graph shows the opposite type of behavior in the progeny of 56 C 11, a sibling to 56 C 1. 56 C 11 seems to transmit good resistance to its progeny. The ELISA test was performed on half of the cross 77 F but no new information was gained from this test: only one plant was found positive but it was already visibly degenerating. Contrasted to 56 C 1, 56 C 11 has steadily increased its production each year.

We should briefly describe the grapes produced. The F_1 hybrids produce berries that are about twice the diameter of those of Agrá and are all black although the color of those crossed with white *vinifera* is not very intense or stable in the wine. Although the fruit is high in sugar, their taste is usually acid and biting, while their aroma resembles that of *vinifera*. The backcross fruit is from 2-4 times larger than Agrá in diameter, sometimes white or red, less acid and sometimes lower in sugar than the F_1 hybrids. The average production of both the F_1 and the backcrosses is very low being less than 200 g/plant which is probably due more to the short photoperiod of the tropics than hybrid infertility. Heavier producers can be selected that give 1-2 kg/plant at the close planting distances used to test them (1 m^2/plant).

Some data showing how the extreme acidity of Agrá can be easily reduced by backcrossing to *vinifera*:

Code	Type	°Brix	T. acid*	pH	Cross
Agrá	Wild	15.8	32.5	2.79	Betty
18 B 9	F_1	20.9	22.4	3.00	Agrá x Carignane
57 E 39	F_1	22.7	17.1	2.75	Agrá x Aligoté
6 G 1	F_1	24.8	17.1	2.88	Sauvignon blanc x Agrá
22 B 8	F_1	23.9	13.4	3.18	Fernao Pires x Agrá
80 D 17	BC_1	23.4	13.3	3.00	(Sylvaner x Agrá) x F. Colombard
81 B 11	BC_1	24.0	12.7	3.20	(Chardonnay x Agrá) x M. Alex
78 G 9	BC_1	23.2	15.5	3.00	(Green Veltliner x Agrá) x Aleatico
83 C 12	BC_1	18.6	11.2	3.05	(Agrá x Carignane) x Ruby Cabernet
59 D 3	BC_1	17.6	12.2	3.22	(Agrá x Ruby Cabernet) x Carignane
77 F 48	BC_1	21.8	7.2	3.23	(Agrá x Aleatico) x M. Hamburg

* in g tartaric acid/l

Discussion and conclusions

Since some of the F_1 hybrids do transmit resistance when backcrossed to *V. vinifera*, resistance must be determined by dominant genes. Some F_1 hybrids, although apparently resistant themselves, are either not transmitting resistance or are doing so in a reduced proportion. We might call these plants partially tolerant to explain this genetically, we might hypothesize that the three genes postulated by Mortensen have an additive effect, which in the harsher climate of Florida was not evident. In this system, one gene will give a little longer survival time, two genes will result in a partial tolerance typified by 56 C 1, three genes will lead to full tolerance like 56 C 11; this would explain the 50 % ratio of the test crosses with 56 C 11: $^1/_8$ of their progeny would be fully tolerant and $^3/_8$ would be partially tolerant.

We will have the results of the first test of this hypothesis when we see whether the sustainability of 56 C 1 turns out to be 25 % with all the survivors partially tolerant. The existence of partial tolerance would be a burdensome impediment to the production of hybrid grape varieties for the tropics.

A much more favorable alternative would be that Agrá has two distinct resistance mechanisms, one depending on three genes that gives the low sustainability of 56 C 1 and another controlled by one dominant gene giving the 50% ratio of 56 C 11. We will have to wait several more years to resolve this question.

It is feasible to use the resistance of V. *caribaea* to PD and many other diseases to breed resistant hybrid vines useful for the production of wine and table grapes for the tropics. Although there are many problems in selecting for resistance to such a weakly aggressive yet virulent disease in a gentle tropical climate, the lack of a viticultural tradition means that we do not have to satisfy a predetermined taste preference, we have no competition, and grape and wine prices are extremely high.

Literature cited

DAVIS, J. M.; PURCELL, A. H.; THOMSON, S. V.; 1978: Pierce's disease of grapevines: Isolation of the causal organism. Science **19**, 75-77.

FENNELL, J. L.; 1948: Inheritance studies with the tropical grape. J. Hered. **39** (3), 54-62.

FREITAG, J. H.; 1951: Host range of the Pierce's disease virus of grapes as determined by insect transmission. Phytopathology **41**, 920-934.

GOHEEN, A. C.; NYLAND, G.; LOWE, G. K.; 1973: Association of a rickettsialike organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology **63**, 341-345.

-- -- ; RAJU, B. C.; LOWE, S. J.; NYLAND, G.; 1979: Pierce's disease of grapevines in Central America. Plant Dis. Rep. **63**, 788-792.

HEWITT, W. B.; 1958: The probable home of Pierce's disease virus. Plant Dis. Rep. **42** (2).

-- -- ; FRAZIER, N. W.; JACOB, H. E.; FREITAG, J. H.; 1942: Circular 353. Univ. of Cal., Coll. of Agricult., Agricult. Exp. Sta., Berkeley, Cal.

HOPKINS, D. I.; 1973: Rickettsia-like bacterium associated with Pierce's disease of grapes. Science **19**, 298-300.

-- -- ; MORTENSEN, J. A.; 1971: Suppression of Pierce's disease symptoms by tetracycline antibiotics. Plant Dis. Rep. **55**, 610-612.

JIMENEZ ARIAS, L. G.; 1980: El mal de Pierce de la vid, su distribucion en Costa Rica y caracteristicas fenotipicas de su agente causal. Tesis presentada para Licenciado en Fitotecnica, Univ. de Costa Rica.

-- -- ; 1985: Evidencia immunologica del mal de Pierce de la vid en Venezuela. Turrialba **35** (3), 243-247.

-- -- ; DAVIS, M. J.; 1987: DNA probe for detection of Pierce's disease bacterium and other xylem-limited bacteria. Phytopathology **77**, 1769.

JIMENEZ MORA, J. M.; 1982: Determinacion de los hospedantes alternos del mal de Pierce de la vid en Costa Rica, mediante tecnicas immunologicas de adsorcion con conjugados enzimaticos.

MORTENSEN, J. A.; 1968: The inheritance of resistance to Pierce's disease in *Vitis*. Proc. Amer. Soc. Hort. Sci. **92**, 331-337.

PIERCE, N. B.; 1892: The California vine disease. U. S. Dept. of Agricult., Div. Veg. Path. Bull. **2**.

RAJU, B. C.; AUSTIN, C.; GOHEEN, A. C.; 1981: Relative sensitivity of selected grapevine cultivars to Pierce's disease bacterial inoculations. Amer. J. Enol. Viticult. **32**, 155-158.

-- -- ; GOHEEN, A. C.; TELIZ, D.; NYLAND, G.; 1980: Pierce's disease of grapevines in Mexico. Plant Dis. **64**, 280-282.

-- -- ; NOMÉ, S. F.; DOCAMPO, D. M.; GOHEEN, A. C.; NYLAND, G.; LOWE, S. K.; 1980: Alternative hosts of Pierce's disease of grapevines that occur adjacent to grape growing areas in California. Amer. J. Enol. Viticult. **31**, 144-148.

WELLS, J. M.; BOLIGALA, C.; RAJU, B. C.; HUNG, H.-Y.; WEISBURG, W. G.; MANDELCO-PAUL, L.; BRENNER, D. J.; 1987: *Xylella fastidiosa* gen. nov., sp. nov.; Gram-negative, xylem-limited, fastidious plant bacteria related to *Xanthomonas* spp. Intern. J. Syst. Bact. **37** (2), 136-143.