Phytopathol. Mediterr. (2000) 39, 92-99

Pathogenicity of three species of *Phaeoacremonium* spp. on grapevine in California

Khan Adalat, Cameron Whiting, Suzanne Rooney, and W. Douglas Gubler

University of California, Davis, Department of Plant Pathology, One Shields Ave., Davis, CA 95616, USA

Summary. Autoclaved sand was inoculated with *Phaeoacremonium inflatipes*, *P. aleophilum* and *P. chlamydosporum*. Single bud cuttings of grapes of cv. Chardonnay were placed in inoculated sand and incubated for three weeks at 27°C. *P. inflatipes* was isolated from 66%, *P. aleophilum* from 59% and *P. chlamydosporum* from 7% of cultured cuttings. The effect of infection on callusing was also observed. Inoculation with *P. chlamydosporum*, *P. aleophilum* and *P. inflatipes* inhibited callus formation in 22, 62, and 72% of total cuttings respectively. Infection of the cuttings by *P. inflatipes*, *P. aleophilum* and *P. chlamydosporum* significantly (P=0.0001) reduced number of roots, plant height, number of internodes, root elongation and dry weight of the above-ground parts. However no significant (P=0.1969) effect was found on root dry weight. Infection and vascular discoloration was found in spurs of the 'Pinot Noir' and 'Chardonnay' inoculated with *P. chlamydosporum*, *P. inflatipes* and *P. aleophilum* inoculated through pruning wounds. Significant differences were found in extent of invasion in 'Pinot Noir' and 'Chardonnay' in response to inoculation. Invasion of spurs of 'Pinot Noir' and 'Chardonnay' inoculated with *P. chlamydosporum* was significantly more extensive than invasion by *P. inflatipes* and *P. aleophilum* respectively (P=0.0001).

Key words: vascular disease, pruning wound infection, *Vitis vinifera, Phaeoacremonium*.

Introduction

Young vine decline of grapes is an important disease of 1- to 7-year-old vines in some production areas in California. This disease was first reported in 1995 (Scheck *et al*., 1998a) in the north-coast grape-growing area. Since then decline has been reported in all grape-growing regions of California. A decline of young grapevines was reported in Italy as early as 1912 when Petri isolated two strains α and β of *Cephalosporium* and one strain of *Acremonium* from declining vines showing internal brown to black vascular streaking (Petri, 1912). Based on Petri's descriptions, Mugnai *et al*. (1999) placed the α *Cephalosporium* strain and the *Acremonium* strain in *P. chlamydosporum* and *P. aleophilum* respectively. In California, Chiarappa (1959) isolated *Cephalosporium* sp. from the brown-red zones bordering decayed wood of grapevines with symptoms of black measles. Upon inoculation into healthy vines *Cephalosporium* sp. produced discoloration of wood and brown streaks and was suggested to be involved in causing black measles. Subsequent isolation in California from vines with black measles revealed 3 additional species of *Cephalosporium* (W.D. Gubler, unpublished). In South Africa, Ferreira *et al*. (1994) consistently isolated *Phialophora parasitica* from the blackened xylem vessels of young vines with slow decline. Crous *et al*. (1996) placed the *Cephalosporium* sp. isolated from esca diseased grapevine in California and the *Phialophora pa-*

Corresponding author: W.D. Gubler
Fax: +1 530 752 5674
E-mail: wdgubler@ucdavis.edu

rasitica isolated from declining grapevine in South Africa in *P. chlamydosporum*. In 1998, Scheck *et al*. reported that *P. inflatipes*, *P. aleophilum* and *P. chlamydosporum* were the causal agents of young-vine decline in California. These three species, *P. chlamydosporum, P. inflatipes* and *P. aleophilum* were shown to be pathogenic to grape seedlings and 1-year-old rooted grapevine cuttings inoculated by dipping injured roots into spore suspensions. Most recently, Mugnai *et al*. (1999) reported that in different parts of Italy 2-year-old vines were severely colonized by *P. chlamydosporum*. In California field symptoms on artificially inoculated plants were similar to symptoms occurring from natural infection and included: reduced caliper size of the trunk, shortened internodes, reduced foliage, reduced leaf size and dead sections in leaf lamina tissue. Infected plants had dark-brown to black flecking in vascular tissue in cross section (Scheck *et al*., 1998a, 1998b). Darkening of the wood mostly occurred in the older vessels of the innermost ring as described by Bertelli *et al*. (1998). Tylosis and dark gummy masses filled the lumen of the xylem elements. Other symptoms observed in naturally infected vines included poor "graft take" where the graft union between the scion and rootstock was poorly callused, leaving the union weak and loose (Scheck *et al*., 1998a, 1998b). The epidemiology of young-vine decline is still not fully understood. Many theories of possible sources of infection have been hypothesized (Bertelli *et al*., 1998 and Mugnai *et al*., 1999) but how and where new infections occur is still unclear. This study reports findings on the pathogenicity of *Phaeoacremonium* spp. and its infection of grapevine in California.

Materials and methods

Fungal strains, culture conditions and substrate

Isolates used in this study were obtained from California vineyards. *Phaeoacremonium inflatipes* (isolate S10*), P. aleophilum* (isolate C1) and *P. chlamydosporum* (isolate SPC) were freeze dried on potato dextrose agar with 100 mg l^{-1} Tetracycline (PDA-Tet) and maintained in sealed viles at 4°C.

Pathogenicity tests on vine cuttings

Inoculum of *Phaeoacremonium inflatipes (Pin)*, *P. aleophilum (Pal)* and *P. chlamydosporum (Pch)* were revived from freeze-dried cultures onto PDA-Tet. Two-week-old cultures were harvested in sterile water. The concentration of inoculum was adjusted to 10^7 conidia ml^{-1} water. Five litres of inoculum of conidial suspensions of *Pin*, and equal amounts of suspensions of *Pal*, and *Pch* were added to 13,000 cc of autoclaved sand per fungus, giving the equivalent of $4.1x10^7$ conidia g^{-1} of sand for each fungus. Sand and inoculum were thoroughly hand mixed. Sand infested with each fungus was placed separately in three identical replicated plastic containers (20x14x10 cm). Ten surface-sterilized, single-bud, dormant cuttings of 'Chardonnay' were placed in the infested sand in such a way that half of each cutting was under sand and half exposed. Lids were placed on the containers to avoid drying of wood and sand. Containers were placed in an incubator at 30°C constant temperature to induce callusing (Nicolas *et al*., 1992; Winkler, 1965). After three weeks, five random cuttings were removed from each container. Cuttings were washed with tap water for 10 min and then surface-sterilized by dipping in 70% ethanol and flaming. Small sections from the bottom 2 cm of each cutting were removed and plated on PDA-Tet. The plates were incubated at room temperature for two weeks and observed for the presence of *Phaeoacremonium* spp., which was confirmed through morphological appearance of the colony and conidial size and shape as described by Crous *et al*. (1996). The experiment was replicated three times. Isolation results of all three experiments were pooled and were converted to percent values on total isolation.

Effect on callus formation

Data were recorded on each of 20 cuttings from each container. Partial-to-complete growth of callus tissue at the bottom end of the cuttings was considered to indicate a callused cutting. Cuttings that had no callus growth at all at the inoculum exposed cut end were rated as non-callused. The experiment was replicated two times. The data from both experiments were combined and percent callused and non-callused cuttings were determined from the total number of cuttings for each pathogen.

Effect on plant growth under greenhouse conditions

Fifty single-bud dormant cuttings of 'Chardonnay' were placed in the infested sand as previously described. The control treatment consist-

ed of placing cuttings in non-infested autoclaved soil, saturated with sterile water. After three weeks cuttings were removed and planted in autoclaved soil in 5.5x5.5x8.5 cm pots. The air-temperature of the greenhouse was maintained for three months at 28±5°C day and 18°C night with a 12-h day. Plants were watered daily. Twenty plants were randomly sampled after 3 months and the following data were recorded:

disease severity: scored as the length (mm) of black streaking extending from the base of the cutting upward;

plant height: measured as length of current growth from the base of the bud to the top of the shoot;

number of internodes: counted from the first leaf to the last leaf;

maximum root length: on each plant the single largest root was measured;

total number of roots: total number of roots coming out of the cutting was counted. Secondary or branched roots were not counted;

dry weight of the above ground parts: all the current growth including cane and leaves was collected. Fresh weight was taken, folded in aluminum foil and placed in an oven at a constant 50°C until constant weight was reached;

dry weight of roots: all roots were collected. Fresh weight was recorded, the material folded in aluminum foil and placed in the oven at a constant 50°C until constant weight was reached. Data were analyzed using the Proc Glm procedure statistical analysis system (SAS), and means compared by Duncan's multiple range test. Data from two experiments were combined and analyzed together.

Pathogenicity tests on standing grapevines

Cultures of *Pch*, *Pal* and *Pin* were grown on PDA-Tet for two weeks. Spores were dislodged in sterile water with a bent glass rod. The spore suspension was passed through a double layer of cheesecloth and final concentration was adjusted to $1x10^6$ conidia ml^{-1} (Ferreira *et al.,* 1994; Scheck *et al.*, 1998a). Twelve grapevines each of 10-year-old 'Chardonnay' (spur pruned and cordon trained) and 10-year-old 'Pinot Noir' (spur pruned and cordon trained) located at the Viticulture Research Station at UC Davis were selected for inoculation. On March 10, 1999, vines were pruned to 6-8 spurs per vine. Three grapevines were randomly allocated to be inoculated with *Pin*, three with *Pal*, and three with *Pch*. Three vines were left as controls. On each vine half of the spurs were inoculated by injecting 0.2 ml of inoculum 1 cm deep into the pith and half by placing 0.2 ml of inoculum on the freshly cut surface as a droplet. The droplet was spread across the entire wound area with the tip of the syringe. The inoculated spurs were allowed to grow under natural environmental conditions. Control spurs were inoculated with sterile water. Plants were allowed to grow for six months. After six months spurs were removed and symptoms were noted. The extent of vascular discoloration was measured from the point of inoculation. The wood was surface-sterilized by dipping in 70% ethanol and flaming. Sections from the leading edge of discolored tissue were cultured on PDA-Tet. Cultured plates were incubated at 23-25°C for 7-10 days.

Results

Pathogenicity tests on vine cuttings

Table 1 shows that all three species of *Pin*, *Pal* and *Pch* were re-isolated from the cuttings exposed to inoculum in infested sand. *Pin* was isolated from 66%, *Pal* from 59% and *Pch* from 7% of cuttings. All three species of *Phaeoacremonium* were thus able to infect dormant grape wood through cut wounds exposed to infested sand. *Pch* was only

Table 1. Percent isolation of *Phaeoacremonium chlamydosporum, P. aleophilum,* and *P. inflatipes* on potato-dextrose-agar modified with tetracycline (100 mg l^{-1}), from grapevine cuttings inoculated through the infested soil.

Treatment[a]	Percent isolation[b]
P. chlamydosporum	7
P. aleophilum	59
P. inflatipes	66
Control	0

[a] Inoculum of *P. chlamydosporum, P. aleophilum* and *P. inflatipes* and sterile water were hand-mixed with autoclaved sand. Single bud cuttings were placed in the infested and non-infested sand and incubated at 30°C for 3 weeks.

[b] Cultured plates that were found positive for the presence of *Phaeoacremonium* were converted to the percent of the total cultured.

Table 2. Inhibition of callus formation in cv. Chardonnay cuttings by *Phaeoacremonium chlamydosporum, P. aleophilum,* and *P. inflatipes*. Cuttings were exposed to infested sand for 3 weeks.

Treatment	No. of cuttings	
	Total cuttings	Percent callused[a]
P. chlamydosporum	98	77.6
P. aleophilum	98	37.7
P. inflatipes	98	27.6
Control	98	100

[a] Cuttings that produced partial to complete growth of tissue at the lower end were considered to be callused. Cuttings that had no growth at all at the cut end were considered non-callused.

minimally successful in colonizing wood from the soil. Culturing of the non-inoculated controls yielded either clean plates or plates over grown with other microorganisms.

Callus formation

Table 2 shows that all grape cuttings in the control treatment callused. Inoculation with *Pch*, *Pal* and *Pin* inhibited callus formation in 22.4, 62.3, and 72.4% of total cuttings respectively. Control plants had no dead area at the base of the cuttings but were completely callused. In cuttings exposed to inoculum, callus formation was partially to completely inhibited. Infected cuttings exposed to inoculum turned black, dried and died from the base.

Effect on growth under greenhouse conditions

Extent of streaking: there were significant differences (P=0.0001) in the length of dark streaking between control plants and plants inoculated with *Pin*, *Pal*, and *Pch* (Table 3). There was no significant difference in the extent of vascular discoloration between plants inoculated with *Pin*, and *Pal* , or between *Pin* and *Pch*. There was a significant difference between *Pch* and *Pal*.

Plant height: significant differences in plant height were found among plants inoculated with *Pin*, *Pal*, and *Pch* and the controls (P=0.0001). Control plants achieved significantly more growth than plants inoculated with *Pin*, *Pal*, or *Pch* (Table 3). There was no significant difference in plant height among plants inoculated with *Pin*, *Pal*, or *Pch*.

Number of internodes: significant differences (P=0.0001) were found in number of internodes between plants inoculated with *Pin*, *Pal*, *Pch* and the controls (Table 3). There was no significant difference in the number of internodes of plants inoculated with *Pin*, *Pal*, and *Pch* (Table 3).

Number of roots: control plants produced significantly (P=0.0001) more roots than plants inoculated with *Pin*, *Pal*, or *Pch*. There was no significant difference in the number of roots on plants inoculated with *Pin*, *Pal,* or *Pch*.

Root length: the root length of control plants was significantly (P=0.0001) different from the root length of plants inoculated with *Pin*, *Pal*, or *Pch*. There was no significant difference between the root lengths of plants inoculated with *Pin*, *Pal*, or *Pch* (Table 3).

Dry weight of the above ground parts: dry weight

Table 3. Effect of *Phaeoacremonium inflatipes P. aleophilum* and *P. chlamydosporum* on plant height, number of internodes, number of roots, root length, dry weight of above ground parts and dry weight of roots in inoculated grapevine cuttings.

Treatment	Disease Lesion size (mm)[a]	Height (mm)	Number of internodes	Number of roots	Root length (mm)	Dry wt. above ground (g)	Root dry weight (g)
P. inflatipes	20 ab	93 b	6 b	13 b	83 b	0.22 b	0.15 a
P. aleophilum	16 b	89 b	6 b	16 b	84 b	0.24 b	0.14 a
P. chlamydosporum	22 a	79 b	5 b	14 b	91 b	0.23 b	0.17 a
Control	00 c	163 a	7 a	26 a	111 a	0.32 a	0.19 a

[a] Means followed by the same letter are not significantly different.

Table 4. Black discoloration in vascular tissue of spurs of standing grapevine in cv. Pinot Noir and Chardonnay inoculated with *Phaeoacremonium chlamydosporum, P. aleophilum* and *P. inflatipes*. Pathogens were either injected into the pith or applied as a drop of spore suspension (10^6 conidia ml^{-1}) to the cut surface of grapevine spurs.

Treatment[a]	No. of spurs with black vascular streaking (%)[a]			
	'Pinot Noir'		'Chardonnay'	
	Injected	Surface	Injected	Surface
P. chlamydosporum	100	100	88	100
P. aleophilum	75	57	28	22
P. inflatipes	45	13	38	0
Control	0	0	0	0

[a] Percentage calculated from the spurs with black vascular streaking, divided by the total number of spurs, and multiplied by 100.

(Table 3) of the above-ground parts of control plants were significantly (P=0.0001) different from plants inoculated with *Pin*, *Pal*, or *Pch*. There was no significant difference between the dry weights of the above-ground parts of plants inoculated with *Pin*, *Pal*, or *Pch* (Table 3).

Roots dry weight: no significant difference (P=0.1969) was found between dry root weights of control plants and plants inoculated with *Pin*, *Pal* or *Pch* (Table 3).

Pathogenicity tests on standing grapevines

Vascular discoloration

Plants of *Vitis vinifera* cv. "Pinot Noir" and "Chardonnay" inoculated with *Pch*, *Pal* and *Pin* had black discoloration of vascular tissue in inoculated spurs (Table 4). Spurs of cv. 'Pinot Noir" inoculated by injection into the pith with *Pch*, *Pal*, or *Pin* produced black vascular discoloration in 100, 75 and 45% of inoculated spurs respectively. Spurs of cv. Pinot Noir inoculated by surface-application with *Pch*, *Pal* and *Pin* produced black discoloration in vascular tissue in 100, 57 and 13% of inoculated spurs respectively. No discoloration was found in the spurs of the cv. Pinot Noir inoculated with sterile water. Similarly, spurs of cv. Chardonnay inoculated by injection into the pith with *Pch*, *Pal* or *Pin* produced black vascular discoloration in 88, 28 and 38% of inoculated spurs respectively. Spurs of cv. Chardonnay inoculated by surface-application with *Pch*, *Pal*, or *Pin* produced black discoloration in vascular tissue in 100, 22 and 0% of inoculated spurs respectively. Spurs of cv. Chardonnay inoculated with sterile water remained clean.

Plants of *V. vinifera* cv. Pinot Noir and Chardonnay inoculated with *Pch*, *Pal*, or *Pin* produced black discoloration of pith tissue of inoculated spurs (Table 5). Spurs of cv. Pinot Noir inoculated by injection into the pith with *Pch*, *Pal*, or *Pin* produced black discoloration of pith tissue in 37, 62 and 33% of inoculated spurs, respectively. Spurs of cv. Pinot Noir inoculated by surface application with *Pch*, *Pal,* or *Pin* produced black discoloration in pith tissue in 12, 37 and 25% of inoculated spurs, respectively. No discoloration was found in the pith of spurs of cv. Pinot Noir inoculated with sterile water. Similarly, spurs of cv. Chardonnay inoculated by injection into the pith with *Pch*, *Pal,* or *Pin* produced black discoloration in the pith in 55, 11 and 11% of inoculated spurs respectively. Spurs of cv. Chardonnay inoculated by surface-application with *Pch*, *Pal,* or *Pin* produced black discoloration in pith tissue in 33, 11 and 0% of inoculated spurs, respectively. The pith of spurs of cv. Chardonnay inoculated with sterile water was clean. Spurs surface-inoculated with *Pin* did not show vascular streaking. Natural senescence of pruning wounds occurred in all treatments and vascular discoloration began immediately below the dead tissue. Vascular discoloration was similar in both 'Pinot Noir' and 'Chardonnay' regardless of whether pith injection or wound-surface inoculation were employed. In transverse sections, symptoms consisted of a group of black vessels in the vascular tis-

Table 5. Black discoloration in pith tissue of spurs of standing vines of cv. Pinot Noir and Chardonnay inoculated with *Phaeoacremonium chlamydosporum, P. aleophilum* and *P. inflatipes*. Pathogens were either injected into the pith or applied as a drop of spore suspension to the cut surface of grapevine spurs.

Treatment	No. of spurs with black pith tissue (%)[a]			
	'Pinot Noir'		'Chardonnay'	
	Injected	Surface	Injected	Surface
P. chlamydosporum	37	12	55	33
P. aleophilum	62	37	11	11
P. inflatipes	33	25	11	0
Control	0	0	0	0

[a] Percent spurs with black pith tissue were calculated from the spurs with black pith tissue, divided by the total number of spurs, and multiplied by 100.

sue, or scattered individual streaks of black vessels, or a partial to complete circle of black vessels around the pith. The black vessels were found adjacent to the pith and around the pith. In the most severe cases, 2-3 layers of black vessels surrounded the pith. White ray cells radiating from the pith towards the cambium separated the blackened vessels. The black discoloration was always more prevalent near the point of infection. All three fungi were able to move from the pith to vascular tissue and also from vascular tissue to the pith. The pith tissue turned dark, dry, and compact, and in some cases the pith developed cavities. One-side necrosis of vascular tissue was common in spurs inoculated with *Pal* and *Pin* but no such necrosis was observed in spurs inoculated with *Pch*.

Fungal isolation.

All three species of *Phaeoacremonium* were positively re-isolated from inoculated spurs of 'Pinot

Table 6. Percent isolation of *Phaeoacremonium chlamydosporum, P. aleophilum* and *P. inflatipes* from inoculated spurs of standing vines cv. Chardonnay and Pinot Noir. Pathogens were either injected into the pith or applied as a drop of spore suspension to the cut surface of grapevine spurs.

Treatment	Isolation (%)[a]			
	'Pinot Noir'		'Chardonnay'	
	Injected	Surface	Injected	Surface
P. chlamydosporum	88	77	88	88
P. aleophilum	55	44	25	12
P. inflatipes	44	0	37	12
Control	0	0	0	0

[a] Percent isolation of *P. chlamydosporum, P. aleophilum* and *P. inflatipes* from inoculated spurs calculated as number of positive over total number of spurs.

Table 7. Progress of streaking (cm) from the point of inoculation, caused by *Phaeoacremonium chlamydosporum, P. aleophilum* and *P. inflatipes* in the inoculated spurs of grapevine cv. Chardonnay and Pinot Noir. Pathogens were either injected into the pith or applied as a drop of spore suspension to the cut surface of grapevine spurs.

Treatment[a]	Length of streaking (cm)			
	'Pinot Noir'		'Chardonnay'	
	Injected (cm)	Surface (cm)	Injected (cm)	Surface (cm)
P. chlamydosporum	6.5 a[a]	8.5 a	3.5 a	3.8 a
P. aleophilum	0.5 c	0.7 c	0.8 bc	0.4 b
P. inflatipes	3.4 b	4.3 b	1.2 b	1.0 b
Control	0.0 c	0.0 c	0.0 c	0.0 b

[a] Each data point is an average of 9 spurs. Means followed by the same letter are not significantly different.

Noir' and 'Chardonnay', except for *P. inflatipes* applied to the surface in 'Pinot Noir' (Table 6). In 'Pinot Noir' *Pch*, *Pal* and *Pin* were reisolated from vascular tissue from 88, 55 and 44% of the spurs inoculated by pith injection, and from 77, 44 and 0% of spur vascular tissue inoculated by surface application respectively. Similarly, in 'Chardonnay', *Pch*, *Pal* and *Pin* were reisolated from vascular tissue from 88, 25 and 37% of spurs inoculated into the pith, and from 88, 12 and 12% of spurs inoculated by surface application respectively. Culturing from the non-inoculated control spurs yielded either clean cultures or were overgrown with microorganisms other than *Phaeoacremonium* spp.

Length of vascular discoloration

Significant differences were found in the length of vascular discoloration of spurs inoculated with *Pch*, *Pal* and *Pin* (Table 7). *Pch*, injected or applied to the wounded surface, caused significantly longer streaks than *Pal* or *Pin* in both 'Pinot Noir' and 'Chardonnay'. *Pal*, injected or applied to the wounded surface, caused the least vascular discoloration. There was no significant difference in lengths of vascular discoloration produced by *Pal* and *Pin* injected or applied to the wounded surface of cv. Chardonnay.

Discussion

All three species of *Phaeoacremonium* were reisolated from the *in vitro* inoculated cuttings cultured on PDA-Tet. The fungi affected the ability of the cutting to callus. Our results agree with those of Ferreira *et al.* (1994) who found that only 29% of inoculated vines callused as compared to 76% in controls. All three species of *Phaeoacremonium* significantly affected plant height, number of internodes, total number of roots and leaf dry weight. Plant height was reduced both by the number of internodes and the length of the internodes. Gummy occlusions were found in the xylem tissue of infected grapevines. The intensity of the slow decline may be related to the number of vessels infected or blocked. The total number of roots was significantly reduced by all three species. Fungal infection resulted in increased lesion size and a reduced living portion or surface area from where roots could be produced. The total dry weight of the roots was not significantly different between control and inoculated plants. The study confirmed that the three species of *Phaeoacremonium* are pathogens of grapes and cause significant damage to young vines. *Pch*, *Pal* and *Pin* were able to infect grapevine through pruning wounds, producing a black vascular streaking similar to what is observed in the field. *Pch* was found to be a more aggressive colonizer of grapevine pruning wounds than to *Pal* or *Pin*. Similar results have been reported from Italy (Mugnai *et al.*, 1999) where field inoculation experiments with esca showed that *Pch*, and to a lesser extent *Pal*, colonized the wood of apparently healthy grapevines. *Pch* has been found in association with measles

and shown to be pathogenic in California but was discounted as the pathogen causing measles (Chiarappa, 1959). However, in Europe (Larignon and Dubos, 1997 and Mugnai *et al*., 1999) have demonstrated pathogenicity and association of *Phaeoacremonium* with esca. One of the many proposed theories regarding the source of infection (Crous *et al*., 1996; Mugnai *et al*., 1999) is that the infection comes from infected mother vines. In a recent study in California, no internal vascular discoloration or presence of species of *Phaeoacremonium* was detected in non-inoculated current-year growth taken from 21 rootstocks (W.D. Gubler, unpublished). In our study it was found that infection of inoculated wood extended only 8.5 cm in spurs inoculated with *Pch* after 6 months. In Italy (Mugnai *et al*., 1999), black streaking extended 30 cm in both directions from the point of inoculation in a 6-year-old grapevine inoculated in the trunk with *Pch*. In California (Chiarappa, 1959), the black streaking extended 10-15 cm in both direction from the point of inoculation in a 7-year-old grapevine inoculated in the trunk with *Pch*. Based on these findings it is unlikely that mother plants serve as a source of inoculum because the movement of the pathogen from the point of inoculation was very slow. Six months after inoculation, *Pch* could be reisolated up to 6 cm away from the point of inoculation (Chiarappa, 1959). The significance of the present study was to confirm that pruning wounds play an important role by providing an avenue of infection for species of *Phaeoacremonium* in California. During the process of grapevine production, each rootstock is wounded at both ends when it is removed from the mother vine, disbudded at two sites, and injured during grafting. It is important to note that *Pch* produces pycnidia on the surface of grapewood when incubated on agar (Whiting and Gubler, unpublished). The spores produced by *Pch* pycnidia were similar in size and shape to conidia produced in culture, and they germinated readily on agar, developing normal colonies. It is more likely, though not yet proved, that *Pch* produces pycnidia on the cane, cordons or spurs of grapevines in vineyards. The conidia produced from these pycnidia infect rootstock through the cut end and wounds produced by disbudding. This suggests that of the three species *Pch* is the most capable of acting as an aerially disseminated pathogen. The other two species seem to be more suited for survival in the soil and in fact do so for long periods of time (Scheck *et al*., 1998b). Further research is needed to determine the potential site of pycnidia production and the role of pycnidia as a source of inoculum in young-vine decline and black measles in California vineyards.

Literature cited

Bertelli E., L. Mugnai and G. Surico, 1998. Presence of *Phaeoacremonium chlamydosporum* in apparently healthy rooted grapevine cuttings. *Phytopathologia Mediterranea*, 37, 79-82.

Chiarappa L., 1959. Wood decay of the grapevines and its relationships with black measles disease. *Phytopathology*, 49, 510-519.

Crous P.W., W. Gams, M.J. Wingfield and P.S.van Wyk, 1996. *Phaeoacremonium* gen. nv. associated with wilt and decline diseases of woody hosts and human infections. *Mycologia*, 88, 786-796.

Ferreira J.H.S., P.S. Wyk Van and E. Venter, 1994. Slow dieback of grapevine: association of *Phialophora parasitica* with slow dieback of grapevines. *South African Journal of Enology and Viticulture*, 15, 9-11

Larignon, P. and B. Dubos, 1997. Fungi associated with esca disease in grapevine. *European Journal of Plant Pathology*, 103, 147-157.

Mugnai, L., A. Graniti and G. Surico, 1999. Esca (black measles) and brown wood-streaking: Two old and elusive diseases of grapevines. *Plant Disease*, 83, 404-418.

Mugnai L., G. Surico and A. Esposito, 1996. Microflora associata al mal dell'esca della vite in Toscana. *Informatore Fitopatologico*, 46(6), 49-55.

Nicholas P.R., A.P. Chapman and R.M. Cirami, 1992. Grapevine propagation. *In:* Viticulture. Vol 2-Practices. (Combe. B.G., P.R. Dry, eds.), Winetitles, Adelaide, USA, 1-22.

Petri L., 1912. Osservazioni sopra le alterazioni del legno della vite in seguito a ferite. *Stazioni Sperimentali Agricole Italiane*, 45, 501-547.

Scheck H.J., S.J. Vasquez, D. Fogle, and W.D. Gubler, 1998a. Grape growers report losses to black-foot and grapevine decline. *California Agriculture,* 52(4), 19-23.

Scheck H.J, S.J.Vasquez, W.D. Gubler and D. Fogle, 1998b. Three *Phaeoacremonium* spp. cause young grapevine decline in California. *Plant Disease*, 82, 590. (Abstrat).

Winkler, A.J. 1965. General viticulture. University of California Press., Berkeley and Los Angeles**,** CA, USA,. 633 pp.