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Differential susceptibility of three grapevine cultivars to *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora* in California

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Summary. One-year-old wood of grapevine cv. Thompson Seedless, Grenache, and Cabernet Sauvignon were pruned and the pruning wound immediately inoculated with a spore suspension (approximately 1×10^6 spores ml^{-1} of water) of *Phaeomoniella chlamydospora* or *Phaeoacremonium aleophilum*. Based on the incidence of esca 3 years after inoculation, Thompson Seedless was rated as the most susceptible of the 3 cultivars. The results are consistent with the observed incidence of naturally-occurring esca in California vineyards on these three cultivars.

Key words: *Vitis vinifera*, esca, Petri disease.

Introduction

Esca, a disease of mature grapevines, has been observed in California vineyards since the 1920s (Hewitt, 1952). Petri disease, a malady that affects young grapevines, was first reported in the North Coast production areas of California in 1995 (Scheck *et al.*, 1998a). Three organisms have been consistently associated with esca and Petri disease in California vineyards: *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum*, and *Phaeoacremonium inflatipes*[1] (Scheck *et al.*, 1998b; Eskalen and Gubler, 2001). Typical symptoms of esca include the presence of purple to brown spots on berries and necrosis between the veins and on margins of leaves which develops into a tiger-striped pattern (Mugnai *et al.*, 1999). Symptoms of Petri disease include the presence of spots or streaks in the vascular tissues, stunted growth, small and chlorotic leaves, and shoot dieback (Khan *et al.*, 2000). The incidence of esca and Petri disease are increasing in California vineyards; however, there is no effective control measure available at the present time. The use of cultivars with field resistance may be an important approach in the management of these diseases. The present study was conducted to determine differences in incidence of esca in 3 grapevine cultivars inoculated with *Phaeomoniella chlamydospora* strain P99.28, *Phaeoacremonium aleophilum* strain P99.10, and *Phaeoacremonium aleophilum* strain P99.4 (previously identified as *Phaeoacremonium inflatipes*).

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[1] Isolates that were previously identified as *Phaeoacremonium inflatipes* are now classified as *Phaeoacremonium aleophilum*, based on results of recent morphological and molecular tests (Rooney-Latham *et al.*, 2004).

Materials and methods

The study was conducted at the University of California Field Research Station in Davis using own-rooted grapevine cv. Thompson Seedless, Grenache, and Cabernet Sauvignon. The cultivars were 15, 15 and 5 years old, respectively, at the start of the study. One-year-old spur wood (1 per vine for cv. Thompson Seedless and Cabernet Sauvignon, 3 per vine for cv. Grenache) were pruned in October 1999 and immediately inoculated by depositing 0.1 ml of spore suspension (approximately 1×10^6 spores ml^{-1} water) of *Pa. chlamydospora* P99.28, *Pm. aleophilum* P99.10, or *Pm. aleophilum* P99.4, on the pruning wound. The fungal isolates used were previously obtained from grapevines showing symptoms of Petri disease in California. Control vines were inoculated with sterile distilled water. Four months after inoculation, one spur from 5 inoculated vines was excised several centimeters from the point of inoculation and brought to the laboratory for observation of the presence of vascular discoloration and for reisolation of the pathogen. Vascular discoloration was observed by visual inspection after peeling the bark. For pathogen isolation, pieces of wood were dipped in 0.5% NaOCl for 3 minutes, rinsed twice in sterile distilled water, plated onto Potato-dextrose-agar amended with tetracyline and observed for presence of the organisms after 7–10 days of incubation at 24±2°C. Differences in susceptibility of the cultivars were determined by yearly assessment of the occurrence of symptoms on inoculated vines.

Results

Four months after inoculation, vascular discoloration was observed in all the sampled spurs inoculated with *Pa. chlamydospora* and *Pm. aleophilum* but not in the control spurs. All the inoculated organisms were recovered from the margins of the vascular discoloration. Two years after inoculation, typical symptoms of esca were observed in 18, 27, and 20% of vines inoculated with *Pa. chlamydospora* P99.28, *Pm. aleophilum* P99.10, and *Pm. aleophilum* P99.4, respectively. None of the control vines of cv. Thompson Seedless or inoculated vines of Grenache or Cabernet Sauvignon showed any symptom at this time. The data on symptom occurrence three years after inoculation are presented in Table 1. On Thompson Seedless, all the vines inoculated with the fungi showed symptoms of esca. More than 70% of the vines inoculated with any of the fungal isolates showed symptoms on fruits only while more than 20% developed symptoms on both leaves and fruits. None of the vines inoculated with *Pm. aleophilum* and only 2% of vines inoculated with *Pa. chlamydospora* exhibited foliar symptoms alone. Symptoms on fruits and leaves are shown in Fig. 1. On leaves,

Table 1. Incidence of esca on three grape cultivars three years after inoculation of pruning wounds with spore suspension (approximately 1×10^6 spores ml^{-1} water) of an isolate of *Phaeomoniella chlamydospora* and two isolates of *Phaeoacremonium aleophilum*.

Cultivar	Fungi	No. of vines (spurs inoculated per vine)	No. of vines with symptoms (% with symptoms)		
			On leaves only	On fruits only	On leaves and fruits
Thompson Seedless	*Pa. chlamydospora* P99.28	45 (1)	1 (2)	34 (76)	10 (22)
	Pm. aleophilum P99.10	45 (1)	0	34 (76)	11 (24)
	Pm. aleophilum P99.4	45 (1)	0	35 (78)	10 (22)
	Control	25 (1)	0	0	0
Grenache	*Pa. chlamydospora* P99.28	8 (3)	0	0	0
	Pm. aleophilum P99.10	8 (3)	0	0	1 (12)
	Pm. aleophilum P99.4	8 (3)	0	0	0
	Control	8 (3)	0	0	0
Cabernet Sauvignon	*Pa. chlamydospora* P99.28	45 (1)	0	0	0
	Pm. aleophilum P99.10	45 (1)	0	0	0
	Pm. aleophilum P99.4	45 (1)	0	0	0
	Control	25 (1)	0	0	0

symptoms initially appeared as small chlorotic spots between the veins and along the margins. These spots later coalesced and became necrotic giving the typical tiger-striped pattern of esca on leaves. On fruits, brown to purplish spots started to appear on the epidermis in the middle of summer, after berries had attained full size. On Grenache, only 1 vine inoculated with *Pm. aleophilum* P99.10 showed symptoms of esca after the third year. The symptoms appeared on both the leaves and the fruits. Symptoms on leaves resembled those of Thompson Seedless while on fruit, the only abnormality observed was shriveling of the berries (also a severe form of symptom observed in California). There have been no symptoms observed on Cabernet Sauvignon up to the present time.

Discussion

Phaeomoniella chlamydospora and *Pm. aleophilum* induced vascular discoloration in all the cultivars and were reisolated from the margins of the discolored vascular tissues. The incidence of esca, however, varied among cultivars indicating that factors other than presence of the pathogen may be involved in symptom expression. Of the cultivars used, Thompson Seedless is apparently the most susceptible with 100% of inoculated vines showing symptoms 3 years after inoculation. It should be noted that vines of Cabernet Sauvignon are younger than the other cultivars used and the absence of external symptoms may be related to age of the vines. Nevertheless, observations in California vineyards showed that esca is general-

Fig. 1. Symptoms of esca on leaves and fruits of grape cv. Thompson Seedless 3 years after inoculation of pruning wounds with *Phaeoacremonium aleophilum* P99.10.

ly more prevalent or evident in Thompson Seedless than in Grenache or Cabernet Sauvignon. Cultivar differences in susceptibility to the 3 causal organisms of esca in Italy – *Pa. chlamydospora*, *Pm. aleophilum*, and *Fomitiporia punctata* - were also observed (Sparapano *et al.*, 2001). Based on the speed of wood colonization and appearance of leaf symptoms, cv. Italia was rated more susceptible to esca than cv. Matilde and the result was consistent with the observed incidence of esca in commercial vineyards in Italy. While *F. punctata* has been isolated in some samples received at our laboratory for routine diagnosis, its role in esca disease in California is still not understood nor fully recognized.

The results obtained show that differences in susceptibility to esca exist among cultivars and demonstrate the potential of artificial inoculation of standing vines in screening for resistance.

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