Further Evidence that *Eutypa armeniacae*—not *Phomopsis viticola*—Incites Dead Arm Symptoms on Grape

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ABSTRACT

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Pruning stubs on grapevine cordons, inoculated in 1975 with mycelial cultures of *Eutypa armeniacae, Phomopsis viticola,* or plain agar, were harvested in 1980. Symptom expression and reisolation of the pathogens confirm that only *E. armeniacae* is capable of inducing the pruning wound cankers and chlorotic, stunted spring foliage that have historically been associated with "dead arm" disease of grape, previously attributed to *P. viticola* in North America.

Investigators in eastern North America earlier this century described the symptomatology and etiology of dead arm disease of grape in considerable detail (3,11,12), but their attempts to fulfill Koch's postulates by inducing the development of stunted chlorotic spring growth, dead arms, and pruning wound cankers were largely inconclusive. The ubiquitous presence of *Phomopsis viticola* Sacc., which has been clearly shown to incite shoot, leaf, rachis, and berry infection (5,12), confused the picture, and the additional symptoms on grape were invariably but unjustifiably attributed to *P. viticola* as well.

In the late 1920s, Coleman (3) ran detailed pathogenicity experiments with *P. viticola* spores applied to the stubs of freshly cut arms of grapevines, but his final conclusion was that these inoculations led to "the inception of what promises to develop into typical (pruning wound) lesions"; definitive proof was not forthcoming. Reddick's experiences (11) were also inconclusive, because his experiments lacked adequate control vines. Nevertheless, the hypothesis that *P. viticola* caused all of the symptoms that are associated with dead arm persisted for more than 60 yr, despite the fact that chemical control measures were effective in controlling only the shoot and leaf necrosis (A. J. Braun, *personal communication*).

The association of *Eutypa armeniacae* Hansf. & Carter with pruning wound cankers, dwarfed shoot growth, and tattered small leaves has recently been demonstrated in all of the major grape-producing areas of North America and numerous other regions of the world (1,2,4,6–9,13), and proof of pathogenicity is published (10).

This paper demonstrates that *P. viticola* is not capable of inducing pruning wound cankers and dwarfed spring foliage of grapevines.

MATERIALS AND METHODS

Grapevines for inoculation. In 1975 nine 8-yr-old grapevines of *Vitis vinifera* L. 'Grenache' growing in a vineyard at the University of California, Davis, were

used for *E. armeniacae* and *P. viticola* inoculations. These vines were healthy and had no visibly weakened or dead arms. They had been trained to a bilateral cordon with six arms on each of two cordon branches. The vines were severely pruned in November by cutting every second arm back to a 1–2 cm stub, which exposed wounds about 2–3 cm in diameter.

Mycelial inoculations with *P. viticola*, *E. armeniacae*, or agar control were made directly onto the freshly exposed 3-yr-old or older wood stubs at three sites on one cordon branch. The three treatments were randomly distributed over the nine vines. Thus, 18 wounds were inoculated with each of the three treatments, providing 54 pruning-wound plots.

Inoculum. Fourteen-day-old cultures of *P. viticola* or *E. armeniacae* on potato-dextrose agar (PDA) were used; the *P. viticola* isolate came from a *Phomopsis*-affected Thompson Seedless vine and the *E. armeniacae* isolate from a *Eutypa*-affected Grenache vine, both growing in a Davis vineyard. The *P. viticola* isolate was sporulating at the time of inoculation; *E. armeniacae* takes several weeks to sporulate.

Pieces of agar plus mycelium were cut to conform to the size of the pruning wounds and applied to the exposed sapwood surfaces with the mycelium toward the wood. Agar alone was applied to the controls. The wounds then were carefully covered with aluminum foil to hold the inoculum in position and prevent rapid desiccation. Aluminum foil was removed after 1 mo.

Assessment of inoculation results. In May of each growing season for 1976-1980 the foliage area immediately adjacent to each pruning site was examined for shoot growth and development and for abnormal leaf symptoms. In May 1980, the cordons from each vine were removed for detailed examination of canker development and to permit laboratory isolations. Shoots, arms, and bark tissues were removed from each cordon. The treated sites then were split longitudinally to permit measurement of vascular necrosis extending away from the original pruning wounds and to facilitate reisolation from the inner wood tissues.

After surface sterilization of the wood pieces by dipping them briefly in a 0.5% sodium hypochlorite solution, a sharp, sterile knife was used to expose a clean surface along the margin of discolored

Table 1. Summary of occurrence of Eutypa dieback symptoms adjacent to sites inoculated with *Eutypa armeniacae* and *Phomopsis viticola* 4.5 yr after inoculation

Inoculation	Total	No. of isolations of *E. armeniacae*	Chi-square value[a]
E. armeniacae	18	13 (72%)	
None (control)	18	3 (17%)	11.36**
E. armeniacae	18	13 (72%)	
P. viticola	18	3 (17%)	11.36**
P. viticola	18	3 (17%)	ns
None (control)	18	3 (17%)	ns

[a]** Significantly different at $P \leq 0.01$; ns = not significantly different.

Table 2. Recovery of *Eutypa armeniacae* and *Phomopsis viticola* from grape stubs 4.5 yr after inoculation

Inoculation	Inoculated	No. of sites from which *Eutypa* isolated	Chi-square value[a]
E. armeniacae	18	17 (94%)	
None (control)	18	8 (44%)	10.46**
E. armeniacae	18	17 (94%)	19.26**
P. viticola	18	4 (22%)	
P. viticola	18	4 (22%)	
None (control)	18	8 (44%)	2.13 ns

[a]**Significantly different at $P \leq 0.01$; ns = not significantly different.

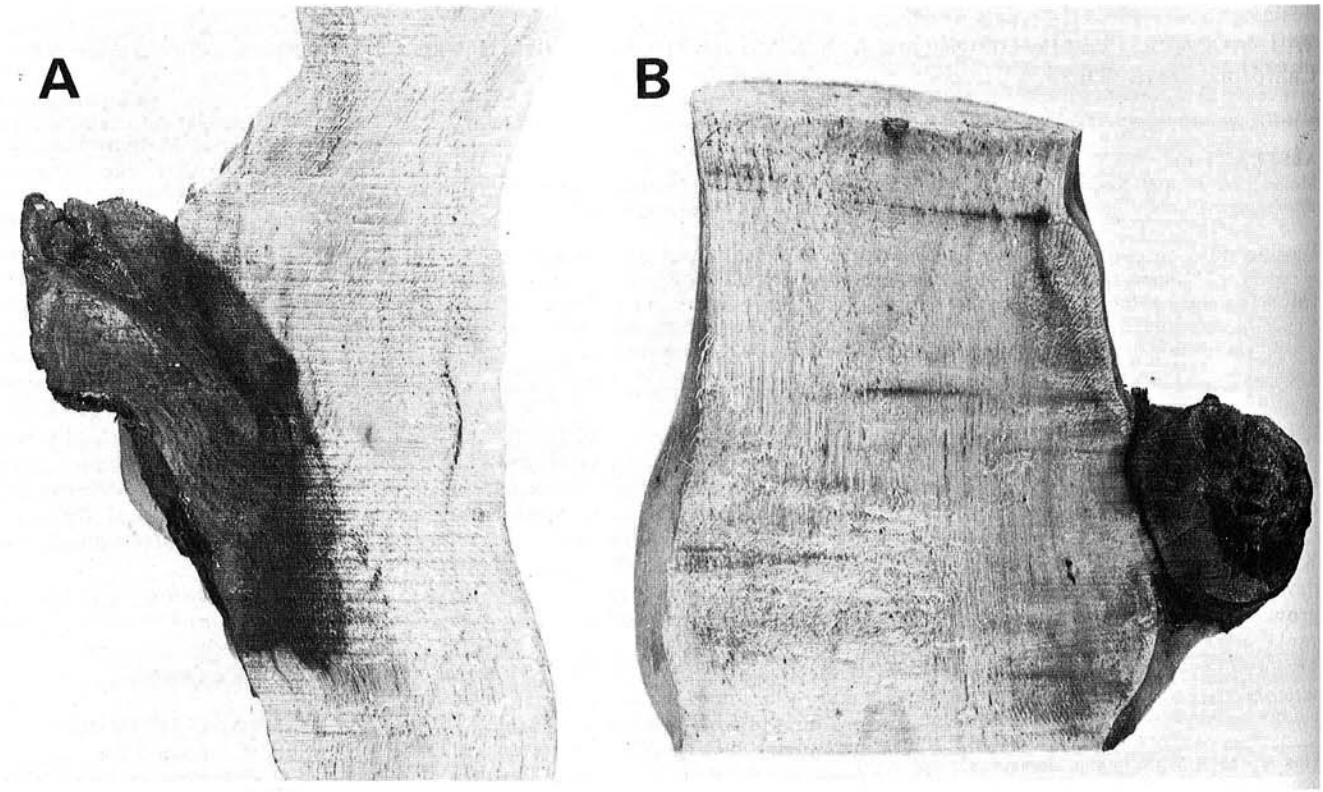

Fig. 1. Grapevine pruning stubs (4.5 yr old) with typical extent of invasion of healthy vascular tissue and incipient canker development: **(A)** *Eutypa armeniacae*. **(B)** Control, which is identical with *Phomopsis viticola*.

vascular tissue; small wood chips (about 10 mm long and 2 mm thick) were cut from this margin, transferred to plates of PDA, and incubated at 20–25 C. If present, colonies of *P. viticola* or *E. armeniacae* were recognizable by mycelial characteristics in 3–4 days.

The observations were tested for expected frequencies by application of the chi-square test, which was adjusted for one degree of freedom by using Yates' correction for continuity. Duncan's multiple range test was used for mean separation.

RESULTS

For the first 2 yr after inoculation, there were no observable effects. Rainfall was low, and not even typical leaf or shoot spotting due to *Phomopsis* was detected. However, in the spring of 1978, which was characterized by late spring rains, *Phomopsis* symptoms became apparent on leaves and shoots proximal to the site of their original inoculation on four of the 18 *Phomopsis*-inoculated arms. Weak, stunted shoots with small, chlorotic leaves (symptomatic of Eutypa dieback infection [10]) also were visible on two of 18 sites inoculated with *Eutypa* and three of 18 inoculated with *Phomopsis*. Two of 18 control sites also showed Eutypa dieback symptoms, indicating that some natural infection occurred in these vines.

By May 1980, 4.5 yr after inoculation, complete assessment was possible, and the cordons were removed. Immediately before removal, symptoms of weak, stunted shoots with dwarfed, chlorotic leaves were recorded on 13 of 18 sites inoculated with *Eutypa*, on three of 18 inoculated with *Phomopsis*, and on three of 18 controls (Table 1). Symptoms of small (0.5–1.0 cm), black, leaf and shoot lesions (*Phomopsis* shoot and leaf necrosis) were noted on one of 18 sites inoculated with *Eutypa*, on nine of 18 inoculated with *Phomopsis*, and on one of 18 controls. Comparison of the numbers of observations positive for Eutypa dieback for *Eutypa*-inoculated sites vs. control showed a significantly ($P \leq 0.01$) greater expression than might be expected due to chance. This relationship also was true for observations of *Eutypa* vs. *Phomopsis* inoculations. When controls were compared with *Phomopsis* inoculations, the frequency of positive observations was identical and obviously was not significantly different, but too few observations were made for a valid chi-square test.

E. armeniacae was reisolated from 17 of 18 *Eutypa*-inoculated pruning stubs, four of 18 *Phomopsis*-inoculated stubs, and eight of 18 controls (Table 2). The frequency of reisolation of *E. armeniacae* was significantly ($P \leq 0.01$) greater when *Eutypa*-inoculated sites were compared with the agar-inoculated control or the *Phomopsis*-inoculated sites. On the other hand, there was no significant difference in the frequency of the reisolation of *E. armeniacae* comparing *Phomopsis* inoculations with controls. *P. viticola* also was reisolated from two of 18 *Eutypa*-inoculated pruning stubs, six of 18 *Phomopsis*-inoculated stubs, and one of 18 controls. Since the expected frequencies of *P. viticola* reisolations were less than five, valid chi-square tests could not be done.

The mean length of invaded, necrotic wood tissue, measured from the original cut, was 74.9 mm for *Eutypa*-inoculated stubs, significantly greater ($P \leq 0.05$) than for *Phomopsis*-inoculated (41.2 mm) and control (49.0 mm) stubs, lengths that were not statistically different from each other (Table 3). Figure 1 shows the typical extent of invasion of healthy xylem by *Eutypa* and incipient canker development, in contrast to that of the control or *Phomopsis* treatment.

Table 3. Length of vascular necrosis per arm (from the surface of inoculated stubs on 18 inoculated arms) 4.5 yr after inoculation

Inoculation	Mean length of dead tissue (mm)[a]
Eutypa armeniacae	74.9 a
Phomopsis viticola	41.2 b
None (control)	49.0 b

[a] Means followed by a common letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

DISCUSSION

Evidence from this experiment further confirms that *P. viticola*, although responsible for some of the symptoms described earlier for dead arm of grapes, is not capable of inducing wood cankers and chlorotic and dwarfed leaves, which are precursors of dead arms and cordons on vines. Thus it is evident that two distinct diseases were included initially in North American literature under the name dead arm.

Reasons are not clear for such prolonged confusion. *E. armeniacae* has an extremely protracted disease cycle, and earlier workers may not have waited long enough for development of symptoms in their pathogenicity tests; furthermore, the asexual state of *E. armeniacae* (*Cytosporina*) is quite nondescript in culture for many weeks, and pycnidium-like bodies usually are produced only after 6–8 wk of incubation. When they do form, the so-called pycnidiospores, which do not germinate, bear marked morphological resemblance to the β spores of *P. viticola*.

In addition, since *P. viticola* is not ubiquitous on vines in the experimental area, the foregoing data indicate that it is capable of long-term saprophytic survival on grapevines. Although *P. viticola* did not invade healthy xylem tissue as did *E. armeniacae*, *P. viticola* persisted for 4.5 yr in the stubs of many wounds where it had been introduced by inoculation.

As observed previously (10), *E. armeniacae* invades grape tissue slowly. Our data indicate that spring symptoms on developing shoots and leaves adjacent to an inoculated pruning stub may appear only after 4–5 yr following inoculation. Even then, vascular necrosis may extend only a few centimeters into healthy wood, although shoot symptoms may appear some distance away. The possible involvement of toxin(s) in the Eutypa dieback disease syndrome awaits further elucidation.

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