

**California Grape Rootstock Improvement Commission (CGRIC)  
American Vineyard Foundation  
Viticulture Consortium  
California Table Grape Commission  
UCDANR-CCGPVE**

**PROJECT REPORT (2001)**

**Project Title:** Study of the Endophytic Nature of *Phaeoacremonium* and *Phaeomoniella* spp. in Grapevines.

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**SUMMARY PROJECT REPORT (2001)**

Some microorganisms can inhabit the internal tissues of a plant without causing apparent harm. These organisms are called endophytes. Some endophytes, however, are capable of rapid growth and of causing physiological disturbance of the plant under certain circumstances, causing disease. *Phaeoacremonium* and *Phaeomoniella* spp associated with Petri disease (Young Vine Decline) have been observed to behave as latent endophytes, capable of inhabiting the internal vine tissues without causing disease symptoms. It is important to establish what are the environmental and physiological factors responsible for triggering their pathogenic behavior. Field observations suggest that conditions of stress for the vines are involved in inducing the occurrence of disease.

Our research project investigates the endophytic biology of *Phaeoacremonium* spp and *Phaeomoniella chlamydospora*, focusing on the capacity of penetration and invasion of grapevine tissues and on the effect of stress factors in the pathogenic response of these fungi.

Isolation results and scanning electron microscopy observations showed that *P. inflatipes* and *P. aleophilum* are capable of penetrating uninjured roots and shoots. Light microscopy observations of paraffin embedded sections showed that rapid spread of the

fungus in injured-inoculated roots was through the vascular tissues and intercellular spaces of the cortex. In injured-inoculated shoots, rapid spread apparently occurred through the intercellular spaces of the pith. Spores of *P. aleophilum* were observed in the xylem vessel. This is the first documentation of the presence of spores of *Phaeoacremonium* spp in the xylem of a grapevine with young vine decline.

Artificial inoculation of grapevines with *P. inflatipes* in greenhouse experiments has shown that the fungus did grow into the shoots although it was abundant at the stem tissue right below them.

Field experiments have been set up to study the effect of different stress factors on predisposing the plants to Petri disease. Stress factors being evaluated are: root configuration, early fruiting, fertilizer treatments, and water deficit stress. These are long-term experiments that will be evaluated over several years. In the first year of application, two foliar and one soil drench fertilizer treatments did not have an effect on the vegetative growth of Merlot/101-14 vines.

The composition of plant vascular sap is known to change under stress conditions. We have compared the effect of sap from water stressed vines on the growth of *Phaeoacremonium* spp and *Phaeomoniella chlamydospora* with the effect of sap from non-stressed vines. The experiments indicated that sap from water stressed vines enhances growth of *P. inflatipes* in vitro, with respect of sap from non-stressed vines. There was no differential effect on the growth of *P. chlamydospora*.

### **Objectives:**

1. Conduct histological investigation on penetration and establishment of infection of *Phaeoacremonium inflatipes*, *P. aleophilum*, and *Phaeomoniella chlamydospora* in roots and shoots of grapes.
2. Determine the effects of water stress as a predisposing factor contributing to young vine decline.
3. Study of the endophytic biology of *P. inflatipes*, *P. aleophilum*, and *P. chlamydospora*.
4. Determine the importance of fruiting age and nutrition as stress factors affecting susceptibility to young vine decline.

## **Justification and Importance of Research:**

Petri Disease (Young Vine Decline) is a widespread problem in newly planted vineyards in California causing economic losses at vineyard establishment. Decline of young vines is associated with infection of roots by fungal pathogens, including *Phaeoacremonium aleophilum*, *P. inflatipes*, and *Phaeomoniella chlamydospora*. These fungi have also been repeatedly isolated from asymptomatic vines. However research programs in our laboratory and other groups worldwide have demonstrated that these fungi are pathogens. When these fungi are inoculated back into grapevines, there is noticeable vine stunting, leaf symptoms resembling water stress, and black or dark brown vascular streaking. Although symptoms may occur overtime in the absence of stress, stress from water deficit, poor planting and early fruiting have been closely associated with disease.

Numerous fungi and other organisms are found inside plants. These organisms are called “endophytes”. The term endophyte, in its most widespread definition, refers to any organism that inhabits the internal tissues of a plant during at least part of their life cycle without causing apparent harm to the plant. Some endophytes seem to go through their lives without ever having a negative effect in their host plants. Others, however, are in fact latent pathogens capable of rapid growth and physiological disturbance of the plant under certain circumstances, thus causing disease symptoms.

We suspect that these pathogens (*P. aleophilum*, *P. inflatipes*, and *P. chlamydospora*) have been present in vineyards for many years, possibly as soil inhabitants and endophytes, but that the planting and re-planting of large acreage has increased awareness of the problem. New rootstocks including 5C, 3309, 110R, 101-14, and Freedom are more susceptible than AXR1. Vigorously growing vines may be able to tolerate low levels of infection but stress due to poor planting techniques, low soil moisture, and early fruiting may predispose vines to infection by decreasing tolerance to these fungi. The biology and epidemiology of this disease in California needs to be worked out so that an effective control program can be developed. Benefits would include the savings of millions of dollars in vineyard downtime and replanting costs.

**OBJECTIVE 1. Conduct histological investigation on penetration and establishment of infection of *Phaeoacremonium inflatipes*, *P. aleophilum*, and *Phaeomoniella chlamydospora* in roots and shoots of grapes.**

### **Experiment 1.**

**Penetration of *P. inflatipes*, *P. aleophilum* and *P. chlamydospora* through uninjured roots and shoots.**

#### Materials and Methods:

Roots free from mechanical injuries were obtained by aseptically growing single-bud cuttings of grapevine cv. Cabernet Sauvignon in culture tubes containing liquid or solid Murashige and Skoog culture medium or in Magenta vessels containing solid medium. Roots were inoculated by replacing the medium with inoculum ( $10^6$  spores/ml of *P.*

*inflatipes* or *P. aleophilum*) for 30 min. Enough spore suspension was poured into the tube to cover the entire root. Roots growing in culture tubes with solid medium were inoculated by injecting 0.5 ml of the spore suspension into the medium around the intact root. For shoot inoculation, plants were inoculated by spraying inoculum on the stem, petiole and lower leaf surfaces with an atomizer. Control plants were inoculated with sterile distilled water. Samples were collected for isolation and scanning electron microscopy (SEM) examination at time intervals after inoculation. For isolation, samples were cut in small pieces and surface-sterilized by dipping them in 0.5% NaOCl for 3 min. The samples were rinsed twice with sterile distilled water, placed on PDA-tet and incubated at room temperature for 10 days. For SEM, sample pieces were fixed in 5% gluteraldehyde in  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  phosphate buffer (pH 7.0) and dehydrated with increasing concentrations of ethanol (10% interval from 10 to 100%, for 30 min each). Samples were then critical-point dried, mounted in aluminum stubs, sputter-coated with gold and examined using a scanning electron microscope.

### Results:

Isolation and SEM examinations showed that the *Phaeoacremonium* spp. tested were capable of penetrating uninjured roots and shoots. In roots, the spores germinated on the root surface within 24 h and formed conidiophores and spores within 48 h (Fig. 1A), as viewed under SEM. Many spores formed germ tubes and spread over the root surface without showing signs of penetration. Penetration, when it occurred, was by the formation of numerous appressoria (Fig. 1B).

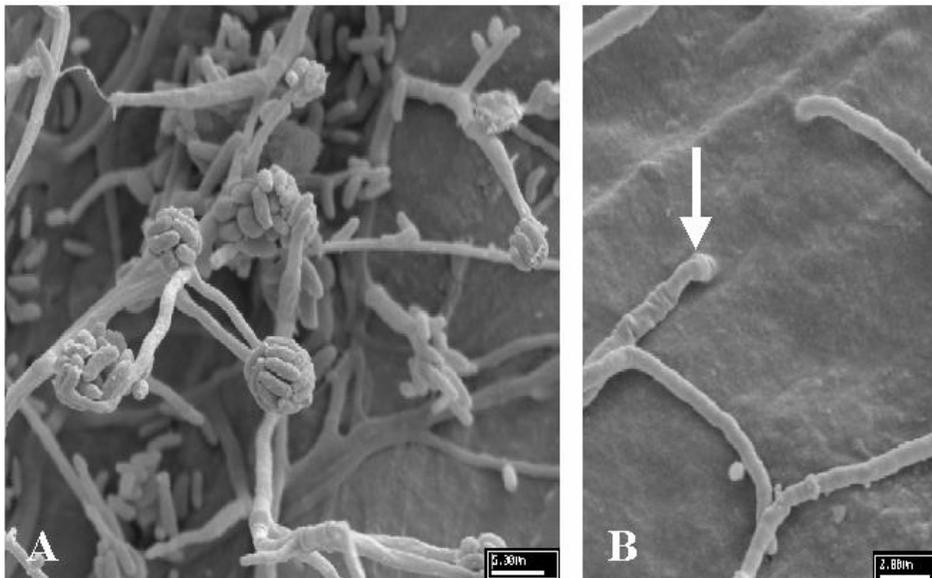


Fig. 1. SEM photomicrographs of *Phaeoacremonium inflatipes* on the root surface 48 h after inoculation showing (A) conidiophore and spores and (B) apparent hyphal penetration via appressoria (arrow).

On leaves, the germ tubes grew randomly, sometimes passing directly over open stomata. Penetration of stomatal pores was never observed (Fig. 2A) although apparent penetration of a guard cell was seen in one occasion (Fig. 2B). Positive isolations were obtained on PDA-tet for both inoculated roots and shoots.

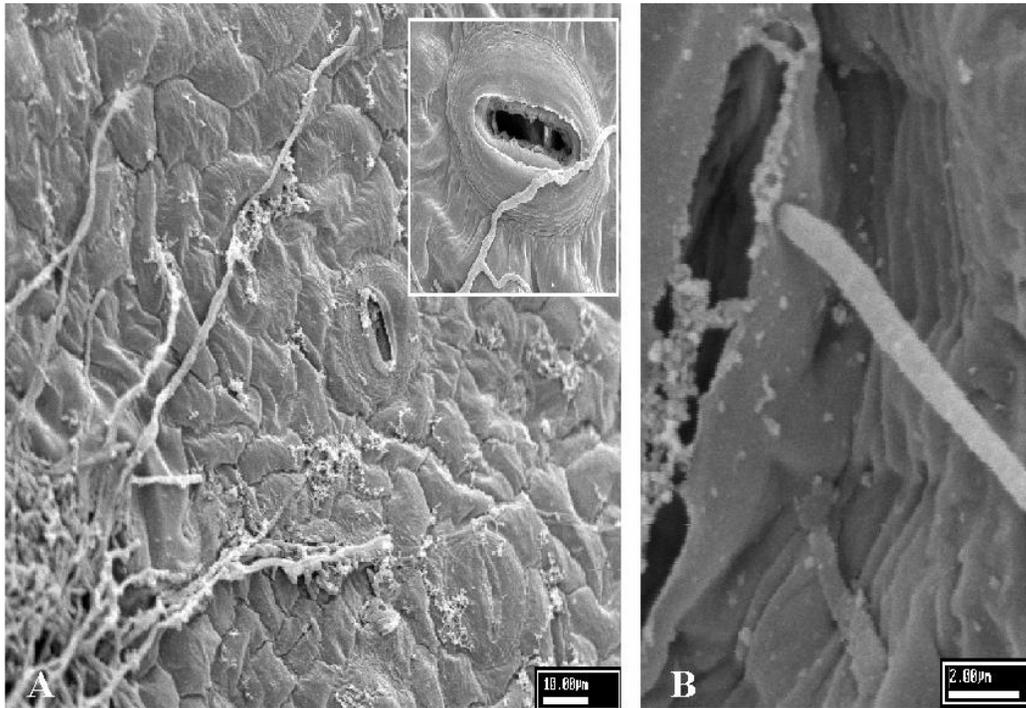


Fig. 2. SEM photomicrographs of the leaf surface 48 h after inoculation with *Phaeoacremonium inflatipes* showing (A) the fungus bypassing the stomatal pore and (B) apparent penetration of the guard cell of the stomata.

## Experiment 2.

### Path of fungal invasion and host response to infection

#### Materials and Methods:

Tissue-cultured grape plants growing in solid Murashige and Skoog culture medium were used. For root inoculation, plants were carefully taken out of the test tube and placed on top of sterile paper towels. About 1.5 cm of the root tip was cut and the cut end was dipped in a spore suspension ( $10^6$  spores/ml of *P. inflatipes* or *P. aleophilum*) for 30 min. The inoculated plants were carefully planted back in the medium. Shoots were inoculated by cutting the shoot tip and depositing a drop of inoculum (approximately 10 µl) on the wound. Two weeks after inoculation, tissue pieces were placed in formalin-alcohol-acetic acid fixative, dehydrated using an automatic tissue processor, embedded in paraffin, cut in 10 micron thickness using a microtome, stained with safranin/fast green and examined under a light microscope. Isolations were also done as described above.

Results:

Course of fungal invasion in injured shoots. Two weeks after inoculation of injured shoot tips, the only symptom of infection was dark brown to black necrosis that extended from the point of inoculation down the stem for 5 mm. Shoot tips of the control plants, inoculated with sterile distilled water, remained green. Light microscopy observations of paraffin sections of inoculated shoots showed that the course of invasion of the host occurred in a similar way for both organisms. At the point of inoculation, extensive hyphal development in the cells and intercellular spaces of all tissues was observed, particularly in the cortex and pith. Some portions of the cortex and pith were completely collapsed as evidenced by their compressed appearance and heavy staining with safranin. In the vascular tissues, some cells were filled to varying degrees with tyloses, gums and/or masses of mycelia.

Upon examination of stem sections remote from the inoculation point, it was apparent that spread of the fungus was initially through the intercellular spaces of the epidermis, cortex, and pith (Fig. 3).

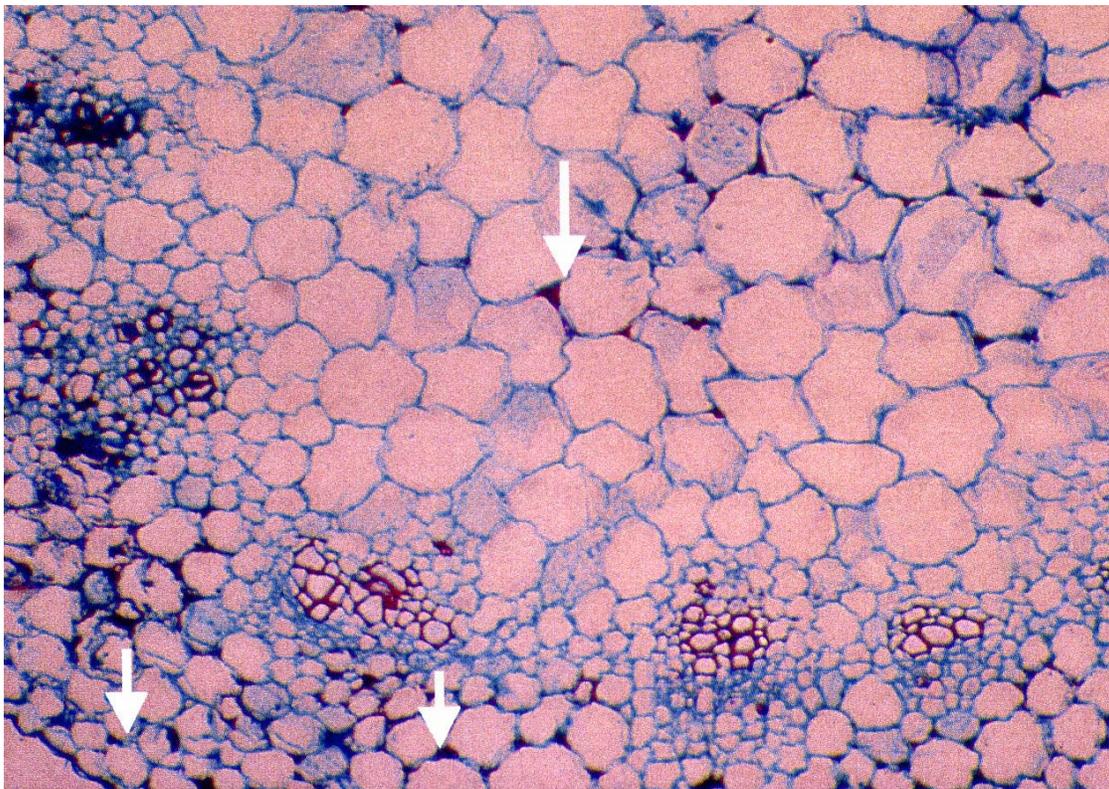


Fig. 3. Cross-section of an infected stem showing spread of the fungus through the intercellular spaces of the epidermis, cortex, and pith (arrows). X = 40.

Rapid spread seemed to occur in the pith where abundant inter- and intracellular hyphae were observed. Formation of conidiophores and spores was also observed in the intercellular space of the pith (Fig. 4).

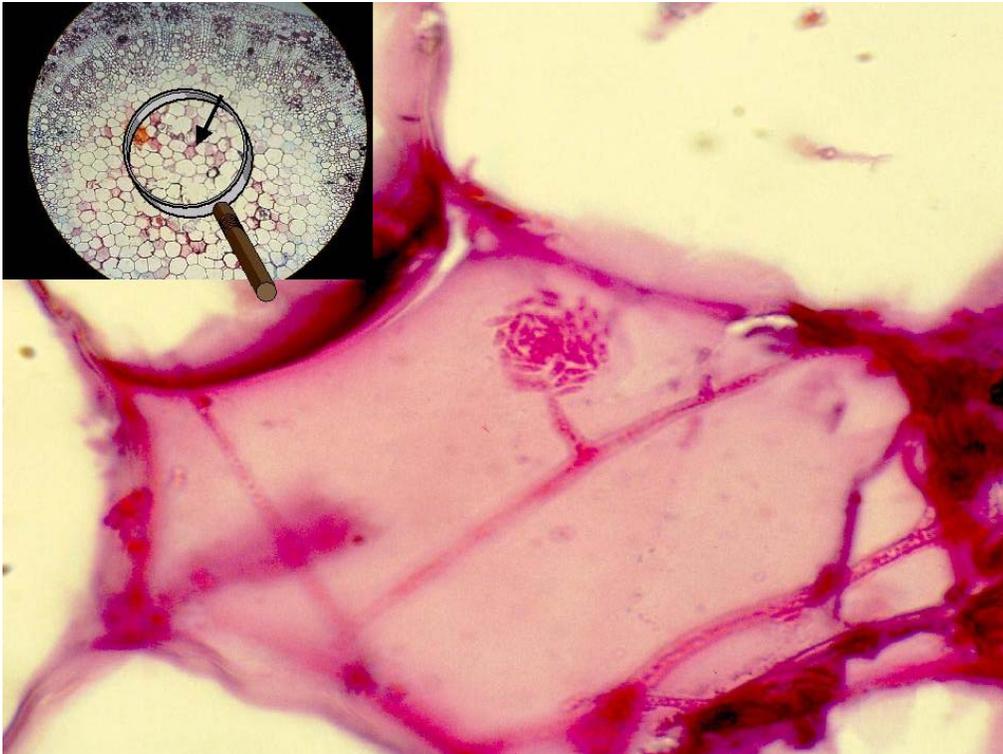


Fig. 4. Cross-section of an infected stem showing conidiophore and spores of *Phaeoacremonium inflatipes* in the intercellular space of the pith. X = 1000.

The xylem and phloem were also invaded but the xylem more extensively. Numerous hyphal strands were observed spreading intracellularly in the xylem vessel (Fig. 5). Some vessels contained tyloses of different sizes and different amounts of gum deposits. Sometimes, gum deposits and hyphae were seen together in a single vessel element. In another section of the infected stem, heavy invasion of the secondary xylem was observed. In this section, numerous hyphal strands were present in the vascular rays and spores were detected in the metaxylem (Fig. 6).

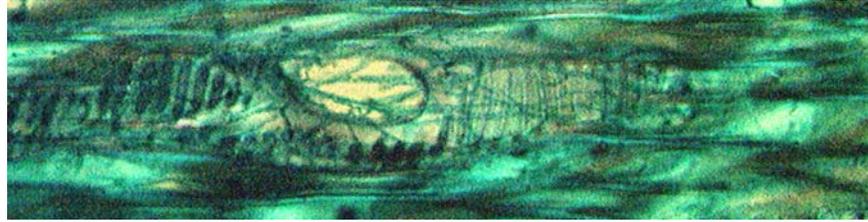


Fig. 5. Longitudinal section of the xylem vessel with numerous hyphal strands. X = 1000.

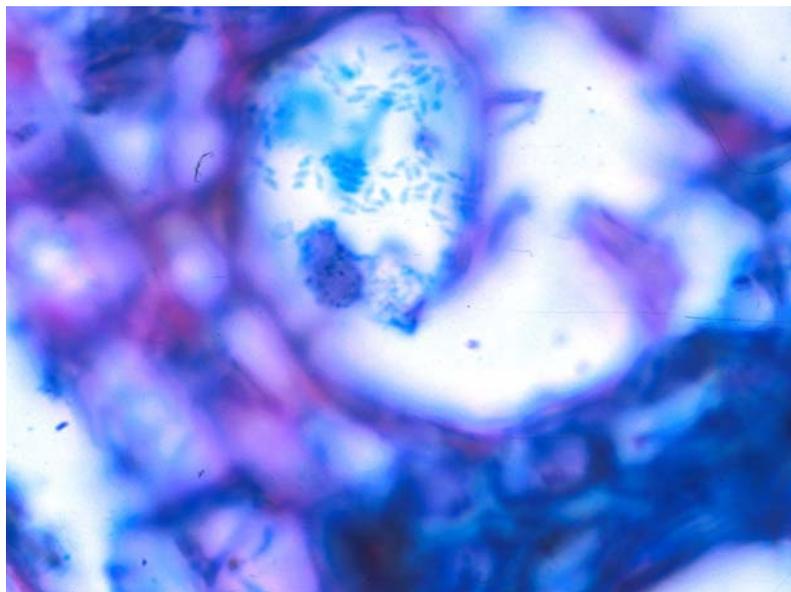


Fig. 6. Cross-section of stem showing fungal invasion in the secondary xylem. Note spores of *Phaeoacremonium aleophilum* in the metaxylem. X = 1000.

Course of fungal invasion in injured roots. Two weeks after inoculation, the roots showed brown discoloration that sometimes extended throughout their entire length. Microscopic examination of samples taken at the point of inoculation showed abundant hyphae in all the root tissues. Rapid spread to other areas was apparently accomplished by way of the vascular tissues and intercellular spaces. Further from the point of inoculation, the fungus was observed travelling in a longitudinal direction in the intercellular spaces of the outer cortical cells. Intracellular hyphae were seen occasionally, but most of the time the only indication of fungal presence was heavy staining of the intercellular spaces (Fig. 7). Gum deposits or tyloses were observed in some vascular tissues, and occasionally both were present in a single vessel (Fig. 8).

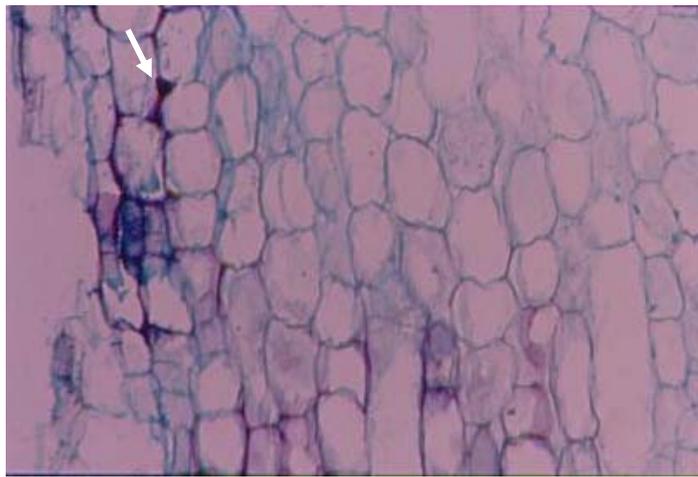


Fig. 7. Spread of *Phaeoacremonium inflatipes* in roots through intercellular spaces of the cortex (arrow). X = 200



Fig. 8. Tyloses (white arrow) and gum deposits (black arrow) in xylem vessel of infected root. X = 100.

### **Experiment 3.**

#### **Histological method of screening for cultivar resistance**

##### Materials and Methods:

Two rootstocks, AXR1 and 110R, rated as least susceptible and most susceptible to *Phaeoacremonium* spp., respectively, (based on greenhouse evaluations using vascular streaking as criterion) will be used in this study. Tissue-cultured plants of these cultivars will be inoculated and samples will be prepared for histological observations to determine differences in periderm, gum, and tylose formation, criteria usually associated with resistance.

**OBJECTIVE 2. Determine the effects of water stress as a predisposing factor contributing to young vine decline.**

**Experiment 1.**

**Effect of soil moisture on young vines inoculated with *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* in a greenhouse environment.**

Materials and methods:

One hundred and thirty three young Chardonnay vines on 3309 rootstocks will be planted in 1gallon pots. Fifty of these vines will have their roots clipped and soaked in a 4L spore suspension ( $10^6$  conidia/ml) for 30 minutes before planting. The suspension will consist of a mixture of the species *Phaeoacremonium aleophilum*, *P. inflatipes*, and *Phaeomoniella chlamydosporum*. Another fifty plants will have their roots clipped and soaked in sterile distilled water for 30 minutes. The extra 33 pots will be used in case that extra leaves are needed to monitor the plant water status

There will be four treatments: (1) inoculated and stressed, (2) non-inoculated and non-stressed, (3) non-inoculated and stressed and (4) non-inoculated and non-stressed. The plants that will not be stressed will be watered daily for the entire experiment. The plants to be stressed will be watered as necessary to maintain the plant water potential status within stress levels (ie. between 10 and 15 bars). Pressure bomb readings will be taken 3 times a week to ensure proper watering.

The following data will be taken: (1) Trunk diameter: at the time of planting, at the beginning of water stress treatment and at the end of the experiment (five months after planting). (2) Shoot length: at the beginning of water stress treatment, and at the end of the experiment. (3) Presence of vascular streaking at different points in the plant: at the end of the experiment. (4) Shoots dry weight: at the end of the experiment.

Results:

This experiment has not yet been completed.

**Experiment 2.**

**Effect of drought stress on symptom development of grapevines inoculated with *P. inflatipes* in greenhouse studies.**

Materials and methods:

A total of 40 one-year old Chardonnay vines on 3309 rootstocks were individually potted in 1-gallon pots on May 30<sup>th</sup>, 2001. Twenty plants were inoculated with *P. inflatipes* by clipping the root tips and soaking them in a spore suspension ( $10^6$  conidia/ml) before planting. The remaining 20 plants were used as controls and their roots clipped and dipped in sterile distilled water. The vines were grown in a greenhouse for a total of 6 months.

Half of the inoculated plants and half of the control plants were irrigated 3 times a week throughout the duration of the experiment. The remaining plants were irrigated 3 times weekly for 4 weeks; then, they were exposed to a drought period when they were irrigated once a week for the duration of the experiment.

Disease was rated based on dark brown streaking of the vascular system at different distances from the point of inoculation (root tips), and as foliar symptoms. To monitor the pattern of foliar symptoms development in relation to the drought occurrence, all the plants were evaluated when irrigation was discontinued and at the end of the experiment. Vascular symptoms were evaluated at the end of the experiment. One repetition of this experiment has been completed; a second repetition will be done in 2002.

#### Results:

Foliar symptoms characteristic of young vine decline were not noticeable either at the beginning of the drought period nor at the end of the experiment.

Vascular discoloration was observed in the crown, stem and scion of all the inoculated and non-inoculated plants, both watered and non-watered. No discoloration was observed in the shoots.

### **Experiment 3.**

#### **Effect of root configuration at planting time and drought stress on vines inoculated with *P. inflatipes* under field conditions.**

#### Materials and methods:

320 Chardonnay grapevines (160 on 3309 rootstock, and 160 on 110R rootstock) were planted on April 18<sup>th</sup>, 2001, at the Plant Pathology Armstrong field headquarters. The roots of half of each rootstock were tied with twine into a “J” shape with the root tips facing upwards. The rest of the vines were left untied. One-half of each group was inoculated with *P. inflatipes* by dipping the roots into a spore suspension ( $10^6$  conidia/ml) for 30 minutes. The vines were planted in 12 in. diameter holes, in rows 8’ apart, with 3’ between vines within rows. Holes were drilled to cause a slick faced hole that was allowed to dry before planting. The plots were furrow irrigated immediately after planting.

Half of the inoculated and half of the non-inoculated plants of each root configuration treatment (“J” shape and control) have been irrigated regularly throughout the duration of the experiment. The other half of the plants has been exposed to a water stress period by withholding irrigation since the last week in May.

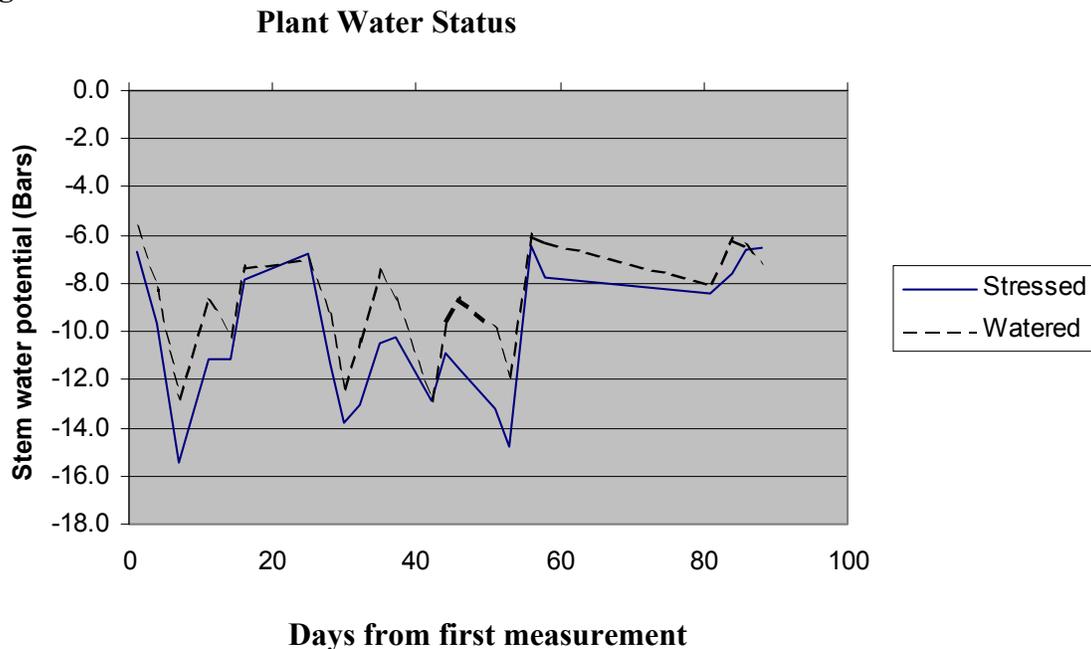
Soil moisture content has been monitored using c-probes. A total of 6 c-probes were placed in the experimental plot: three in the block to be water-stressed, and 3 in the block to be irrigated. Each c-probe contains 6 soil moisture sensors, placed at 10, 20, 30, 40, 60 and 80 cm depth. Sensors will remain on the ground for the duration of the experiment. Plant water potential was monitored by pressure bomb readings taken three times a week.

### Results:

There was no fruit crop on the vines in this first year. Vegetative growth could not be measured because rabbits grazed the shoot tips. However, non-stressed vines were nearly twice the size of stressed vines in terms of shoot length prior to the rabbit invasion. During this 2001 season, foliar symptoms characteristic of Petri disease did not occur.

The water status of the vines was measured as stem water potential (Figure 1). Each data point is the average of five individual leaves sampled from different plants. The first water pressure reading was done on June 25, 2001. The water potential of the stressed vines (solid line) and the irrigated vines (dashed line) follow parallel fluctuations. The vines are considered to be under water deficit stress at -10 to -15 Bars. In this case, water stress did occur and was more pronounced on days 7, 30 and 53. This plot will be evaluated throughout 2002 with caliper ratings, shoot length measurements and foliar symptoms being assessed.

**Figure 1.**



### **OBJECTIVE 3. Study of the endophytic biology of *Phaeoacremonium* and *Phaeomoniella* spp.**

#### **Experiment 1.**

**Progress of the endophytic colonization of root inoculated grapevines by *P. inflatipes*.**

#### Materials and methods:

This experiment was set up as described in Objective 2:Experiment 2. The inoculated vines were used to investigate the advancement of endophytic colonization by *P. inflatipes* and how it was affected by the occurrence of drought stress. The movement of

*P. inflatipes* inside the vines was determined by reisolation of the fungus from different parts of the vine. Isolations were done from 6 different parts of each plant: root tips, crown, upper stem (26 cm above root growth), and scion (right above graft union). From Pi-inoculated plants isolations were also done from lower stem (6cm above root growth) and shoots (6 cm above base). Each sample consisted of a 1 cm long section. The epidermis or bark was stripped off and the rest was surface sterilized in 10% commercial bleach for 1 minute. Shoot sections were treated with bleach for only 30 seconds. The samples were then rinsed in sterile distilled water three times. The samples were cut in half lengthwise, and small pieces were shaved off the cut surface, including areas of vascular discoloration and pith tissue whenever present. Tissue was placed on Difco potato dextrose agar (PDA) amended with 100 ppm Sigma tetracycline (PDA-Tet), and incubated at 22C.

Results:

Colonies of *P. inflatipes* growing on culture medium were identified by microscopy observations. Table 1 indicates the percentage of vines sampled from which *P. inflatipes* was isolated. Data are based on partial results. *P. inflatipes* was isolated from previously inoculated vines. However, this fungus was also recovered from some of the non-inoculated plants indicating that it was either present in the vines, or there was contamination among the plants in the greenhouse. The latter has been shown to occur.

*P. inflatipes* was mostly recovered from scion tissue right above the graft union (Table 1), which was also the area of strongest discoloration. The high frequency of *P. inflatipes* in the scion of non-inoculated plants suggests that this could have been the origin of the contamination. An interesting finding was that *P. inflatipes* was never recovered from shoot tissue despite the fungus being present in the stem tissues right below the shoot.

*P. inflatipes* was recovered from root, crown and lower stem of non-stressed vines but not from the same tissues of water stressed vines. These results suggest a stronger fungal growth or colonization under non-stress conditions than under stress. This interpretation contradicts our hypothesis that conditions of stress for the plant would favor the endophytic growth of *P. inflatipes*. However the data on stressed plants is based on 50% of the plants included in the test, while data of non-stressed vines are based on all 10 plants sampled. Results will be reevaluated upon completion of the data collection.

**Table 1. Isolation of *P. inflatipes* from potted grapevines.**

Plant tissue	Inoculated Stressed	Inoculated Non-stressed	Control (Non-inoculated)
Root	0.0	28.6	0
Crown	0.0	42.9	0
Lower stem	0.0	28.6	na
Upper stem	25.0	14.3	20
Scion	75.0	87.5	80
shoot	0.0	0.0	na

## Experiment 2.

### Effect of sap from water stressed and non-stressed grapevines on the germination and growth of *P. inflatipes* and *P. chlamydospora* in vitro.

#### Materials and methods:

Sap was extracted from 2-year old Chardonnay grapevines on 110R rootstock planted at an experimental field plot in Davis, CA. Sap was extracted from vines that had been regularly irrigated, and from vines that had not been irrigated for one year. The water status of the vines was measured as the stem water potential the same day of sampling. Sap was extracted using a pressure bomb. The sap obtained from each group of vines was pooled and frozen at -20 C until used.

Cultures of *Phaeoacremonium inflatipes*, *P. aleophilum* and *Phaeoconiella chlamydospora* were grown on PDA-Tet at 22 C for 7 days. To prepare each individual spore suspension, an agar plug was removed from the culture plate and placed in a test tube with 10 ml of sap. The tube was vortexed to remove spores from the culture plug. The concentration of spores was adjusted to  $10^4$  conidia/ml using a hemacytometer. Culture plugs were added until the desired concentration was reached. Each suspension was filtered through 5.0  $\mu\text{m}$  filter units and aliquoted into 3 tubes. Similar suspensions were also prepared in sterile distilled water.

The tubes were incubated shaking horizontally at room temperature. Germination and growth of the fungus were recorded after 7 days. The fungal suspension in each tube was poured previously weighted on filter papers. The filters were dried in an oven at 45C for 22 hours and weighted again. Fungal growth was estimated as the difference in weight. This experiment has been done once. A second repetition will be done in the summer of 2002.

#### Results:

The stem water potential of the irrigated vines sampled ranged between -5.8 and -8.2 Bars; the stem water potential of the non-irrigated vines ranged between -11.0 and -16.0 Bars.

Neither *P. inflatipes* nor *P. chlamydospora* conidia germinated in sterile distilled water.

Growth of *P. inflatipes* in sap of stressed vines was significantly ( $p < 0.05$ ) greater than the growth with the same isolate in sap from irrigated vines. This shows that stressed grapevine vascular sap allowed greater growth of *P. inflatipes* than non-stressed sap. While these results are preliminary, they tend to support our original hypothesis that these endophytes can be turned “on” or “off” by changes in sap physiology. This work is continuing. The fact that the same results were not observed for *P. chlamydospora* is interesting and at the same time not surprising because we now know that this species survives as pycnidia on grapevines and probably is not an endophyte but rather a true pathogen capable of infecting through pruning wounds. No differences were observed between of *P. chlamydospora* growth in sap from stressed and non-stressed vines (Table 2).

**Table 2. Differential fungal growth in sap from water-stressed and irrigated vines.**

	Sap stressed	Sap irrigated	Difference	p<0.05
<i>P. inflatipes</i>	4.33	2.33	2.00	***
<i>P. chlamydospora</i>	2.67	2.33	0.33	NS

**OBJECTIVE 4. Determine the importance of fruiting age and nutrition stress factors affecting susceptibility to young vine decline.**

**Experiment 1.  
Fruiting Age.**

Materials and methods:

Allowing grapevines to fully develop berries in the first one or two years after planting is considered to be a debilitating factor that may favor development of Petri Disease. With this experiment we want to follow a newly planted vineyard through four consecutive years to compare the effect of fruit harvesting at different plant ages.

An experimental plot was set up at a commercial vineyard (Chardonnay) planted on July 2000 in Sonoma County. The experiment has been set up as a completely randomized design with 4 replications per treatment, and 10 plants per replication (40 per treatment). Fruits will either be allowed to mature and then harvested, or mechanically dropped at fruit cluster stage. The fruit from a group of vines will be harvested starting the first year of bearing; other groups will be harvested starting each of the following 3 years.

Treatments:

- (1) harvesting fruits in 1<sup>st</sup> year of fruit bearing;
- (2) dropping fruits in 1<sup>st</sup> year and harvesting in 2<sup>nd</sup> year;
- (3) dropping fruits in 1<sup>st</sup>, and 2<sup>nd</sup> year and harvesting in 3<sup>rd</sup> year;
- (4) dropping fruits in 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> year and harvesting in 4<sup>th</sup> year.

Results:

In this first year the vines did not bear fruit. Application of the treatments is expected to start in the current year 2002.

## Experiment 2. Fertilizer Trial.

### Materials and methods:

A fertilizer test plot was established in two commercial vineyards (Merlot/101-14) in Sonoma County that had previously shown vine decline symptoms. One foliar and three soil drench treatments were tested. Treatments are as follows:

- (1) 3:1 (20-1-7) + (10-0-1) Gold'n Gro (soil drench)
- (2) 3:1 (16-0-4) + (10-0-1) Gold'n Gro (soil drench)
- (3) 3:1 (20-0-4) + (10-0-1) Gold'n Gro (soil drench)
- (4) 4.5 oz/acre in 50 gal/acre Messenger (foliar application)
- (5) No applications (control treatment)

Treatments were applied twice in each vineyard. At the Healdsburg plot foliar treatments were applied on May 20 and June 28, 2001; soil drench treatments were applied on May 26 and June 28, 2001. At the Sonoma plot both foliar and soil drench treatments were applied on May 27 and June 28, 2001. Each treatment was replicated 4 times with 10 vines per replication. The effect of treatments on vegetative growth was evaluated by measuring fresh weight of prunings.

### Results:

The results of vegetative growth at the Healdsburg vineyard are shown in Figure 2. There were not statistically significant differences ( $p < 0.01$ ) between any of the fertilizer or control treatments. Each bar is the average weight based on 40 vines measured. Vertical bars represent the standard error of the mean. Vegetative growth at the Sonoma plot is pending evaluation in the coming weeks.

**Figure 2.**

