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Viticulture and grapevine declines: lessons of black goo

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Summary. Diseases that cause the premature decline and death of grapevines are a threat to the economic viability of vineyards everywhere. Although research has brought about major progress in the understanding of grapevine viral diseases, the same progress has not occurred with fungal diseases which can be equally devastating to the expected life span of vineyards. The role of pathogenic fungi in grapevine declines may be overlooked because of misattribution to other causes. For example, decline in grafted vines where the rootstocks are infected with species of *Phaeoacremonium* and/or *Cylindrocarpon* may be attributed to cultural practices or site conditions. Therefore, growers unknowingly plant (and replant) new vineyards with diseased material from nurseries who are unwittingly selling infected plants. Clearly, there can be no solution for a problem that remains unrecognized. Today, there is an urgent need for research into the significance of the spread of disease by *Phaeoacremonium* spp.-infected mother vineyards, particularly in American *Vitis* rootstock varieties. The economic hardship posed by these fungi extends beyond the grower to the nursery industry as well. Even those nursery suppliers who are aware of the potential presence of *Phaeoacremonium* spp. in their plant material do not know exactly what steps to take to eliminate it. Founded in 1998, the International Council on Grapevine Trunk Diseases (ICGTD) can work to improve communication among those studying the same diseases by developing common terminology and data collection protocols, as well as coordinating research projects to avoid redundancies. This multi-disciplinary approach will hasten progress in understanding the significance of pathogenic fungi in propagation material, as well as finding methods of controlling disease expression in infected vineyards. Using the case of black goo caused by *P. chlamydosporum*, both the symptom and disease expression, I offer here my outlook on issues related to the study of grapevine declines.

Key words: esca, black goo, grapevine decline, rootstocks, *Phaeoacremonium chlamydosporum*.

Introduction

Viticulturists are called upon to determine the causes of poor vine growth. They must advise growers both on how to cure existing problems and how to avoid them in the future. Because there are many causes of poor vine growth, one must systematically review all the possibilities in the process of discovering the true cause. During the early 1990s, I saw many cases of poorly growing young vines that were grafted on phylloxera-resistant rootstocks of proven adaptability to the conditions. It was assumed that these vines were suffering from latent viruses or related graft incompatibility. However, this seemed unlikely because the problem was apparent in both certified and uncertified planting stock.

Furthermore, because folletage or apoplexy was at times a manifestation of this problem, I wondered if there could be a relationship to esca disease. Unfortunately, as the many papers in this Proceedings attest, the term "esca" referred to a wide array of symptoms and causal agents, for which there was no apparent common terminology.

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Table 1. Updated summary of "black goo"[a] symptoms found inside woody tissue (from Morton, 1995).

LOCATION **Only in wood > 1 year**	**SYMPTOM** **In cross section**	**COMMENTS**
Black goo under 10x magnifier (all locations)	Dark amber to black color, the consistency of thick honey or yeast extract. Circa 10 minutes after cutting a cross section, one can see shiny domes of tar-like "sap" oozing from individual xylem vessels.	Normal grapevine sap is crystal clear. This would not be confused with the brown vessel staining caused by *Verticillium*.
In roots	Not always present. Goo in the center portion of the structural roots. Not on or directly under the epidermis (with the possible exception of a wound entry site).	Roots with black goo appear normal from the outside. They may be smaller than goo-free roots. Goo most likely found in oldest roots near base of cutting. Rarely do all of the main roots show the symptom.
In rootstock stem American *Vitis* sp.	There are black spots or a blackened sector in a partial or full circle within the oldest annual xylem ring surrounding the pith, and sometimes in the pith itself. The black dots can extend out into other xylem rings, but is never found in the newest xylem tissue. They may show a radial grouping, following medullary rays or sometimes appear in circular fragments, corresponding to annual growth rings. The goo dots tend to be more dispersed, less dense as the vine gets older and new xylem rings form. In older vines, one may or may not see necrotic heart wood.	On the outside, one can sometimes see unhealed wounds at disbudding sites and a longitudinal fissure. Often, where there is sectorial blackening and goo in one place on the xylem ring, there is little subsequent xylem development in that place and normal growth around it leading to uneven xylem development. Wood tissue around the black goo spots may be moist & tan-colored or dry & tan-colored, but it is not dark brown and hard dead tissue as one finds with Eutypa cankers.
At graft union	There may be a black line around the graft itself with black goo dots just below it. There is nearly always a different expression of black goo symptoms in the rootstock and scion tissues at the union and directly above or below it.	A change in symptoms from rootstock to scion may be caused by anatomical differences between the genotypes, or by the physical, swirling maze of vessels where the two vascular systems join together.
In scion trunk *Vitis vinifera*	If *P. chlamydosporum* has entered through pruning wound then the black goo will move downward through vessels associated with the wound. This means that the goo symptom may not necessarily be in the center of the trunk.	With very young vines, the scion trunk above the union often appears normal, even if the rootstock below shows much black goo. Occasionally just above the graft union, there is black pinpoint speckling in the scion xylem rings, always beginning at the pith and working outward. Never in the newest (outer ring) xylem.
In scion arms/cordons	Black goo may be found in the 2+ year wood at spur positions and looks quite similar to infected rootstocks of the same age. The cordon wood between the spurs may be goo-free.	In older vines, the inner xylem can be very dry and may or may not show black goo in the inner xylem rings. This suggests xylem plugging remote to that particular place.

[Note: *Phaeoacremonim chlamydosporum* has been renamed *Phaeomoniella chlamydospora* by Crous & Gams (see their article in this issue). Also Petri grapevine decline has been proposed as a new name for black goo decline.]

A goal of the ICGTD group should be to develop a glossary of terms for the symptoms of grapevine wood diseases in English with synonyms in other languages.

Field symptoms associated with black goo decline

Unlike Eutypa dieback and classic esca/black measles, most of the field symptoms of black goo decline are non-specific and easily attributable to other causes. They include:

– high failure rate in first year of bench grafts or field grafts
– strong growth until late July-August and then sudden collapse

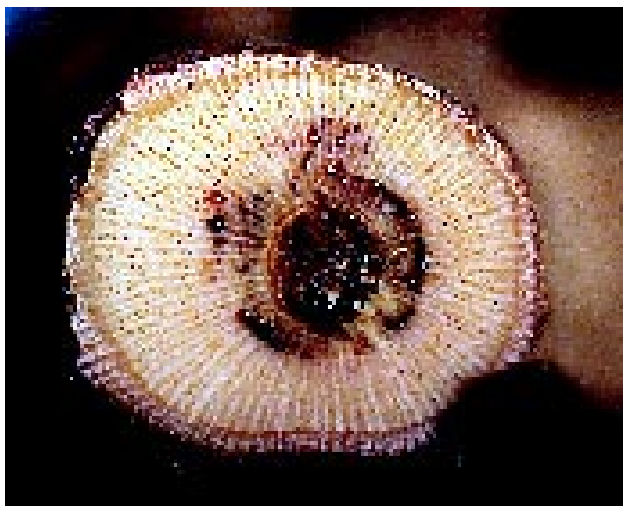


Fig. 1. This vine is a 420A rooting which was field-budded with Cabernet Sauvignon in North Coast California. The graft union was poorly callused. Scion growth was weak. There is already evidence of a fissure visible on outside of the rootstock stem and black goo inside the xylem.

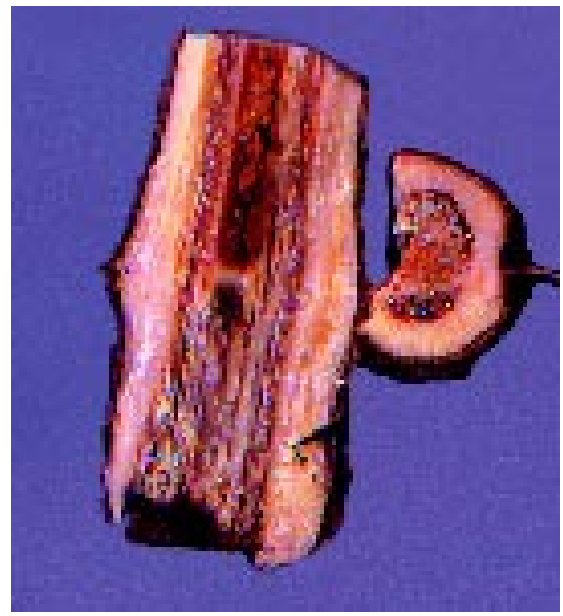


Fig. 2. Black goo in the pith and inner xylem of a 5C rootstock stem.

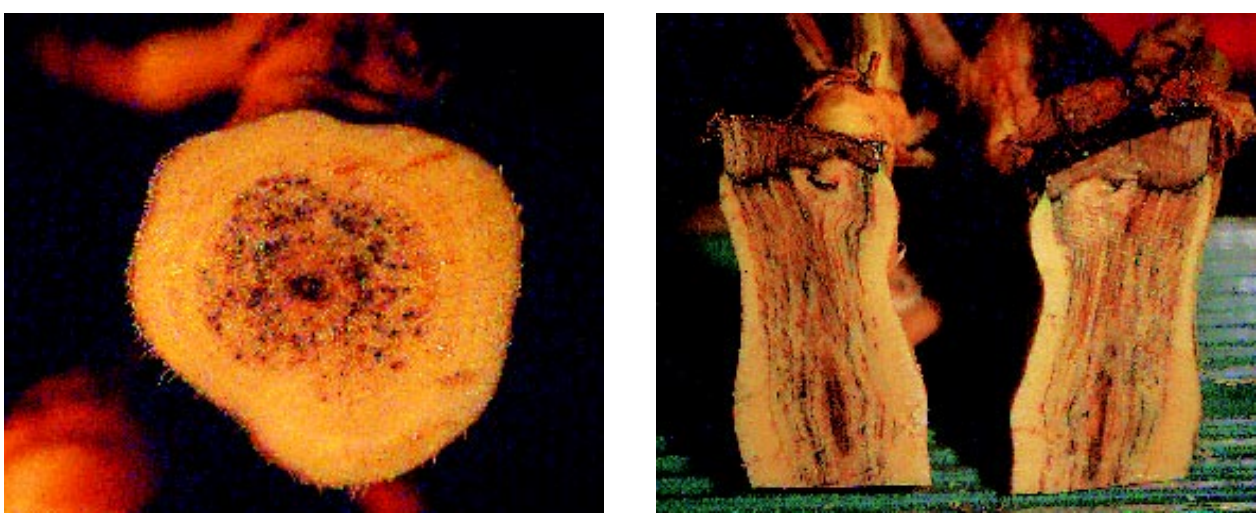


Fig. 3. Black goo caused by *P. chlamydosporum* seen in the cross-section of a circa 13-year-old, certified 420A mother vine in California. Note the healthy tissue in the outer xylem rings (left). Longitudinal view of black goo inside the 420A mother vine in Fig. 3 on the left. Note the heavy black line at the intersection of live with dead wood (right).

– apparently normal growth the first and second year, followed by a failure to increase into an appropriate size for the conditions
– lack of vine-to-vine uniformity in a random pattern
– weak plants at the row ends where they are generally more vigorous
– abnormally low resistance to water stress, low fertility, or high crop loads
– inconsistent disease expression of the same plant from year to year (as with esca/black measles).

Vines infected with *Phaeoacremonium chlamydosporum* are likely to have other diseases and stresses as well: it is reasonable to suppose that these other problems will exacerbate and be exacerbated by the presence of the fungus. Toxins produced by the fungus (Sparapano *et al*., 1998) might cause black goo and tyloses to block normal xylem function and perhaps bring out symptoms associated with:
– nutrient deficiencies (interveinal or marginal chlorosis)
– drought stress (blotchy leaf scorch)
– viruses ("leaf roll")
– overcropping (premature vine shutdown and fruit dessication).

The synergistic effects between *P. chlamydosporum* and other fungi, virus, bacteria, etc. deserve further study.

Terminology issues

Why black "goo" and not "gummosis" or "streak"

In 1994 when I first became concerned about gummy black spots and stripes in the xylem of rootstocks, I looked carefully at all the photos and disease symptoms described in the *Compendium of Grape Diseases* (Pearson and Goheen, 1988). Significantly, "canker" is listed in the glossary of this publication yet "gummosis," "streak," and "stripe" are not. I began calling the symptom "black goo" and published color photos of it in a trade magazine hoping to invite feedback from other viticulturists and scientists (Morton, 1995). Now this black goo has been adopted as a field term by some pathologists as well (Magarey *et al*., 1999).

Goo means sticky like glue. Easy to remember, it is short-hand for more precise terms like "dark-colored, gummy masses filling the lumen of xylem elements" or "tar-like contents inside xylem vessels." Furthermore, the term "black goo" can be useful for differentiating the specific symptom caused by *P. chlamydosporum* from other types of gummosis, gum resins, and tissue blackening caused by different organisms, or unfavorable growing conditions, as well as from the natural gumming associated with wounding.

Petri (1912) established that the wound-related gummosis and brown wood streaking are signs of disease and they are not caused by wounding alone. If wounding were sufficient to cause extensive and debilitating gummosis, the time-honored practice of annual pruning would not be possible, and neither would the life-saving grafting of *vinifera* cultivars onto phylloxera-resistant rootstocks which requires the joining of two wounds!

Further research is needed to distinguish this from similar wood symptoms caused by each species of *Phaeoacremonium*. For example, perhaps other species do not cause the characteristic pinking of surrounding tissue commonly seen with *P. chlamydosporum*? Perhaps the xylem exudate from *P. aleophilum* is more brown than black? Perhaps *P. aleophilum* causes a more rapid death of tissue surrounding the xylem vessels so that the main symptom associated with this species is brownish wood than black goo?

Latin names like *Phaeoacremonium chlamy-*

Table 2. Parts of the vine and their age.

Part of vine	Age
Seedling, green shoots	< 1 year
Cutting, cane, spur	1 year
Green growing plant	1? year
Dormant rooted cutting	2 years
Field grafted vine	Rootstock 3+ years, scion 1+ year
Trunk	Rootstock (1+ non-renewable), scion 1+(renewable)

dosporum and *Fomitiporia punctata* are simply not going to be adopted as disease names by growers. The most useful common names are those that describe the single most distinctive symptom of a disease like powdery mildew for example. Note: almost no one refers to this disease as *Uncinula necator*. This should be remembered when the ICGTD group and others consider new names for the various diseases encompassed by "esca."

For purposes of discussion, see the several definitions from one British dictionary *(Holliday, 1989) and one American glossary of plant pathological terms **(Shurtleff and Averre, 1997). They show the need for a thoughtful yet simple glossary of terms for grapevine declines:

gummosis — *an exudate of gum from a plant, frequently external and from woody tissue; can be used for internal symptoms, e.g. filling of lysogenous cavities or xylem occlusion; **pathologic condition characterized by excessive secretion of sap, "gum," latex, or resin by or in a plant tissue; the products of cell degeneration. May be due to a parasite working within the plant, to unfavorable growing conditions, or to other environmental factors;

streak — *a disease characterized by elongate lesions or areas of discoloration, usually of limited length, on leaves with parallel venation or on stems; **an elongated lesion, usually with irregular sides;

stripe — *a disease characterized by elongate lesions or areas of discoloration, of indefinite length, on leaves with parallel venation or on stems"; **elongated necrosis of tissue between vascular bundles in leaves or stems of cereals and grasses.

Defining age: how old is "young?"

Because the fungal wood decline diseases are progressive they are studied in vines of all ages. The fungi infect vines in a number of ways and the age of vines at the time of infection can vary considerably (Ferreira *et al*., 1994). It now appears that black goo may be one of the first internal symptoms of esca disease (Chiarappa 1959, Mugnai *et al*, 1996).

The resurgence in interest in grapevine decline diseases is partly because they are striking younger and younger vineyards. One reason for our failure to understand the etiology of esca is that, historically speaking, it is a disease that has been studied chronologically backwards — first old vines and then decreasingly younger ones (Chiarappa, 1999). Current research is now being directed toward esca's "prenatal" origins in propagation mother vines.

The youngest vine stage is that of seedlings which are of no practical value in viticulture because of the infinite variations in their genetic status. Micro propagation can be done with tiny amounts of green growing material. Most of the world's millions of vines, however, are propagated from one year cuttings. Among other things, the anatomy of the wood tissue changes with age (Goffinet, 1999). Therefore, terminology used by researchers should clearly indicate the age of the wood they are working with and should reflect common useage by growers (Table 2).

For clarity in communication, it would be helpful if there were a standard vine age terminology that would be useful when discussing decline status. Two suggestions follow (Table 3).

Data collection: links between vineyards and laboratories

Another tool that would help improve communication among researchers and the industry at large would be to develop standards for plant material evaluation and a companion database (see sample grapevine pre-plant evaluation). Such information can be helpful in evaluating whether vines are diseased because they are infected by a pathogen, or simply discolored or weak for non-pathogenic reasons.

Researchers should collect their own samples from the vineyard whenever possible. They can see first-hand why the grower is concerned and be reminded that their work has a direct impact on the success or failure of someone's livelihood. It is useful to see the site conditions, the degree of care the vines are receiving, and, if possible, verify the trueness-to-type of the material because genotype is an important factor.

Visual inspections for wood diseases in the field generally requires some destructive sampling. Performing a "strip-tease" by peeling the bark off the trunk is useful for diagnosing some wood diseases like those caused by species of *Verticillium*, *Phytophthora*, *Armillaria*, *Eutypa*, and *Botryosphaeria*. This will not show the black goo symptom that lies within the outside xylem ring. How-

Table 3. Suggestions for vine age and decline definitions.

Suggestion 1:		
Vine age	Adjective	Decline status
1 to 3 years	New	New planting failure
4 to 10 years	Young	Young vine decline
11 to 20 years	Young mature	Premature vine decline
21 to 50 years	Mature	Vine Decline
51 plus	Old	Old vine decline
Suggestion 2:		
Vine age	Adjective	Decline status
Less than 15 years	Young	Young vine decline
More than 15 years	Mature	Vine decline
More than 50 years	Old	Lucky to be alive!

ever, some clues to black goo decline and esca are visible on the exterior of the vine including: fissure and cracking, out of round stem malformation; small but deep pits formed by uncallused wound and disbudding sites; and poorly callused graft unions. Multiple cross sections of wood provide a chronological snapshot of when the infection took place and how it progressed.

It is important to be very specific when describing all symptoms and their location on the plant: in words, photographs, and line drawings. Photographs bring the vineyard to the pages of scientific journals. Terms like "typical symptoms," "characteristic interveinal-chlorosis" or "stunted growth" should be substantiated with a photograph. Color photos are needed to show the difference between terms like "brown necrosis" and "red-brown wood." An example of excellent graphics may be found in the articles on esca by Mugnai *et al.* (1996, 1999) which are exceptional and understandable to virtually any reader, trained or untrained in plant pathology.

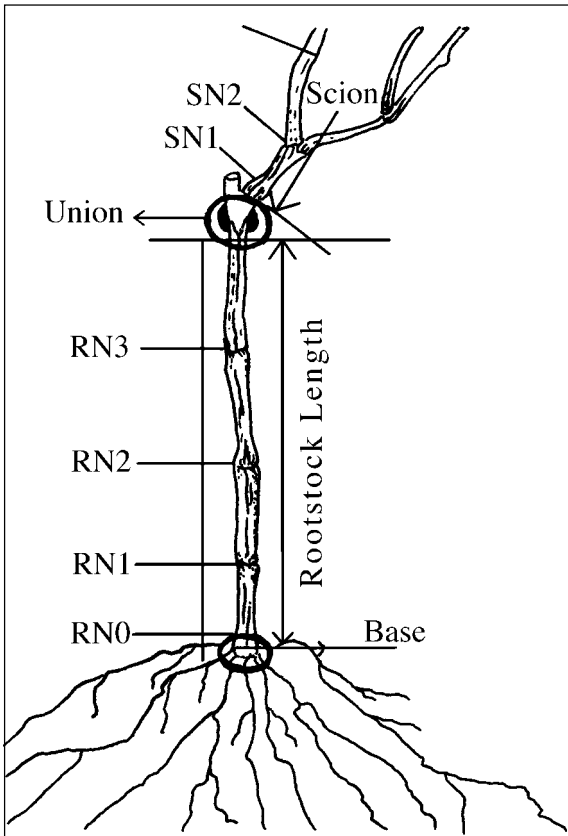
Finally, as foundation and nursery mother blocks age, they become increasingly vulnerable to wood disease organisms. Such blocks are often a favorite source of wood for experimental plantings. Thus, a fundamental question researchers must ask themselves is: What is the health status of the plant material being used in research? I know of at least one experiment that had to be scuttled when all the vines, including the controls were found to be infected with the same fungi used for inoculation. Wood diseases in field trials are a relatively common problem that compromises the potential for long term studies and the integrity of data.

Practical considerations

The past informs the future

How often we forget what we already knew! In 1912, L. Petri reported on the role of three undetermined species of *Cephalosporium* and *Acremonium* (now thought to be *Phaeoacremonium* spp.) in causing what he called brown wood streaking and wood gummosis, and their association with grapevine decline. Yet neither Petri, nor any of his contemporaries followed up on this work. Because Petri subsequently focused his research on *arricciamento*, a decline caused by what is now known as fanleaf virus, his observations on decline caused by fungi were virtually lost to viticulture until 1998 (Mugnai, 1999a; Mugnai *et al.*, 1999b; Graniti *et al.*, 1999). Similarly, L. Chiarappa (1959) demonstrated the pathogenic nature of a species of *Cephalosporium* (currently referred to as *P. chlamydosporum),* but the questions he posed were left unanswered after he abandoned grapes for coconuts and other food crops.

When dealing with a disease of unknown eti-



DRAW CROSS SECTION ABNORMALITIES BELOW
(cracks, goo dots, necrosis, rot)

DRAW PITH-TO-WOOD RATIO →

DRAW ROOT PLACEMENT →

IDENTIFICATION: Date_____ **ID#**:_____
Scion:_____(genotype_____) **Rootstock**:_____(genotype_____)
Photo? / Slide?/Digital Image? Yes / No **Roll-Frame#/File name**_____
VINE TYPE: **Bench graft?** — Green / Dormant **Field Budded?** — Yes / No **Own rooted?** — Yes / No
TOTAL WEIGHT:_____
STRUCTURE: Bent? — Yes / No **Twisted?** — Yes / No **Flat?** — Yes / No **Grooved?** — Yes / No
SCION GROWTH — Strong / Medium / Weak
UNION: **Callus** — Complete / Partial _____%
UNION CROSS SECTION: Clean / necrosis___% / rot____% / dots goo____%
DISBUD SITE CROSS SECTION: Depth — Shallow / Deep-to-pith **Rot?** — Yes / No
ROOTSTOCK TOTAL LENGTH: (Rootstock Node 0 to Union base) _____cm
ROOTS at base # < 2mm _____ # ≥ 2 mm_____
Callus — Partial? / Complete?
Pith visible? — Yes / No
Rotten? — Yes / No
QUALITY RATING:
Plantable — 5-Excellent 4-Good
Borderline — 3-Fair
Unplantable — 2-Poor 1- Half-dead 0-Dead
PATHOGEN ANALYSIS: bacteria ____ fungus(i) ____ phytoplasma ____ virus_____

Fig. 1. Grapevine pre-planting evaluation sheet. RN#=rootstock node position, SN#=scion node position. Researchers could revise this sheet by assigning numerical codes for creating a database system. The principle is that the condition of plant material used in experiments should be systematically evaluated and documented.

ology, the biological clock chimes a different tune for growers than it does for research scientists. Growers and viticulturists must take action today on problems that scientists may not fully resolve for many years. Thus, the following discussion is made with the understanding that some of the suggestions are of potential, but unproven efficacity.

Lessons from viruses: strive for pathogen-free plants

Wood fungal pathogens should become a research priority. Those pathogenic fungi that can be spread through propagation material should be included in state and private certification / registration schemes set up to provide material free of important viruses and virus-like pathogens.

There is an urgent need to study the wood decline organisms specifically in phylloxera-resistant rootstock varieties – both when grown as mother vines and when part of vines grafted in the field.

It is not known yet how to fully guarantee *P. chlamydosporum*-free plants. An obvious starting place is to begin with clean mother stock, but it may take some years until this is possible. The efficacy of hot water treatment (HWT) of 30 to 45 minutes at 50 °C remains a question at this point due to contradictory reports. In any case, additional costs and plant losses associated with HWT will cause some nurseries to seek alternative solutions, while other nurseries may adopt this procedure as routine, especially for rootstocks.

Because the *P. chlamydosporum* sporulates much more abundantly on necrotic tissue than in healthy tissue (Pascoe, personal communication), it has been suggested, but not demonstrated, that green tip propagation might reduce chances for infection. However, without knowing how they got infected, I have seen black goo in young rootstock plantings at virgin-to-grape sites that were planted with vines originally mist-propagated from green shoots.

Common sense would say that poor quality plant material, low starch reserves, dehydration damage, etc., will be more susceptible to fungal diseases. Moreover, strong rooted cuttings – with a high level of starch and low amount of damaged tissue – would seem to be less adversely affected by the presence of *P. chlamydosporum*. Such plants will be less adversely affected by the starch degrading activity of this fungus (Mugnai *et al*., 1999). Perhaps a reduced amount of necrotic tissue might mean lower levels of sporulation and subsequent toxin productions.

This would support the idea that the use of poor quality or mishandled cuttings will increase the failure rate in material infected with *P. chlamydosporum*. The use of strict sanitation measures and systemic fungicides in the nursery process might reduce the risk of spreading the disease from infected to uninfected plants, and perhaps lower the inoculum in infected plants. Free market forces will favor those nurseries who demonstrate concern and some measure of control over the problem of fungus-infected plant material.

Research into the precise modes of infection is urgently needed, particularly the existence of latent infections caused by microconidia in the xylem. These would be difficult to control by any of the methods under discussion at this time.

Barring the door but is the enemy still within?

In vineyards, where there has been a high level of esca-black measles, one would assume that there is *P. chlamydosporum* growing on old wood and root tissue in the ground, although I have not seen confirmation of this. Thus, every possible prophylactic measure should be taken to prevent new plants from coming into contact with the fungus:

– sterile soil immediately around the plants
– pre-inoculation of the plants with *Trichoderma* or other potentially antagonistic organisms
– treating all wounds with fungicides, especially wounds made at the base of the plant near the graft union.

The practice of retraining new trunks might be effective where the *P. chlamydosporum* has entered pruning wounds in the cordon and slowly moved downward. However, if the rootstock is also infected, then does this practice risks doing more harm than good by creating a very large pruning wound? Even if this wound is treated and sealed against fungal invasion from the air, it could still become a fertile field for sporulation of the fungi within.

We have seen the dangers of *P. chlamydosporum* entering a vine from the outside – that is not the whole story. The story will not be complete until we follow that trail of black goo which has been hidden from view, the one that moves from the inside the wood tissue to the outside.

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