Screening V*itis* rootstocks for resistance to Armillaria root disease.

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Armillaria root disease is caused by *Armillaria mellea*, a fungal pathogen with a host range of 500+ plants. It infects the roots of many species of forest trees. When forests are converted to vineyards, the pathogen survives in residual roots (inoculum) and can colonize grapevines up to several years after planting. The pathogen decomposes woody roots, leading to a decline in host capacity and eventual death. Postinfection treatments temporarily improve the productivity of infected vines, but do not significantly decrease mortality rates. Preplant soil fumigation provides control for several years, but replants are eventually infected when their roots reach inoculum that escapes fumigation. Given that *Armillaria* is recalcitrant to these traditional approaches, an alternative is needed.

Because of its broad host range, replanting infested sites with another species is unlikely to eliminate the Armillaria root disease problem completely. However, some species seem to be more tolerant of infection than others and, because the pathogen grows so slowly, replanting with such species is a feasible control option. The problem is that researchers do not have a reliable means of infecting plants in the greenhouse. This is a serious obstacle to identifying resistant plant material. What rootstocks are thought to be ‘resistant’ or ‘susceptible’ typically comes from field observations and anecdotal information. Such information is somewhat unreliable because it is unlikely that all plants were equally challenged by the pathogen and it is not known whether or not ‘resistant’ plants escaped infection simply by not encountering inoculum.

The focus of our current research is to develop a reliable means of infecting greenhouse plants, as this would allow us to identify resistant rootstocks not only for *Vitis*, but also for other crops. We previously screened eight *Vitis* rootstocks in the greenhouse, using an existing and time-consuming technique (Baumgartner and Rizzo 2006). Nonetheless, we identified a range of resistance to infection in the *Vitis* germplasm. Our current objectives are to: (1) identify a quantitative means of evaluating resistance, and (2) develop microsatellite markers to genotype *A. mellea* isolates.

Objective 1. Identify a quantitative means of evaluating resistance. Our approach is to examine the infection rate of roots among *Vitis* germplasm grown in tissue culture. To test the hypothesis that fine roots are susceptible to infection by the pathogen, 2-week-cultures of *A. mellea* were inoculated to the surface of rooting medium in which two rootstocks were grown. Within days, thick cords of the pathogen’s hyphae - rhizomorphs – proliferated in the agar and contacted roots. After 4-6 weeks, inoculated plants wilted and died; noninoculated controls remained healthy. Roots were harvested from both inoculated and noninoculated plants for microscopic examination of infection by *A. mellea*. Intracellular growth of the pathogen among the roots of inoculated plants was confirmed by treating roots with wheat germ agglutinin (WGA) conjugated to a fluorophore (Alexa Fluor 488; Invitrogen, Carslbad, CA), and viewing them under the confocal microscope (Figure 1). WGA is a carbohydrate-binding protein that selectively binds sialic acid and N-acetylglucosaminyl sugar residues, which are concentrated on the plasma membrane.

Objective 2. Develop microsatellite markers to genotype *A. mellea* isolates. Microsatellite markers were developed using the procedure of Glenn and Schable (2005), with restriction enzymes and probes that have proven successful for other fungi (Winton et al. 2007) (Table 1). The primers were labeled and used to screen a subset of 55 *A. mellea* isolates for locus variability. Such markers will allow us to identify unique strains among field isolates, which is not possible with traditional pairing methods. In this way, we will be able to select unique strains of the pathogen for inoculating plants in the greenhouse.

In summary, with confocal microscopy, we can now track colonization of fine roots by the pathogen within weeks of infection. In addition, we can genotype field strains of the pathogen. Alexa Fluor 488-WGA is our best bet for detecting the pathogen in tissue culture plants, but it may not distinguish *A. mellea* hyphae from that of soil-borne, saprophytic fungi on the roots of plants grown in soil. Therefore, we are currently developing a genetic transformation system, in order to create a green fluorescence protein

(GFP)-expressing strain of the pathogen, a procedure that has proven critical for the study of other pathogenic fungi (e.g., *Magnaporthe grisea*, *Sclerotinia sclerotiorum*; Lorang et al. 2001).

References

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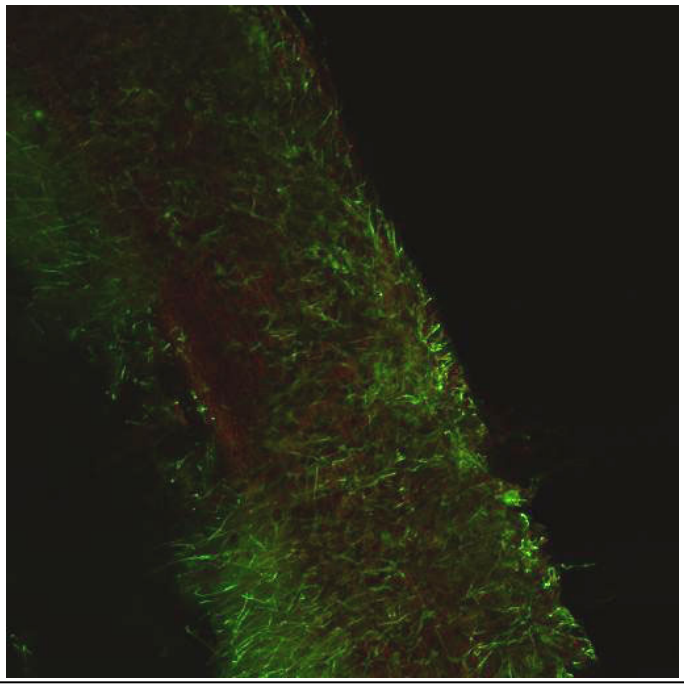

Figure 1. Maximum projection series from confocal microscopy of root tip stained with the Alexa Fluor 488 conjugate of wheat germ agglutinin (WGA). The root is positioned longitudinally; the tip points toward the top of the photo. The bright green hyphae of *A. mellea* are seen in and around the root tip, especially at upper left. The rectangular, root cell walls and cell contents autofluoresce faint green and red, respectively. This root is from a tissue culture plant that died one month following inoculation with *A. mellea*.

Table 1. Microsatellite primers for *Armillaria mellea*

Locus	Motif	F Primer	R Primer	Size (bp)
Am024	CAC	VIC-GACCGGACCTCGTATGACAC	GCACTTTGGTGAAACCATCC	201
Am035	CAC	VIC-GCTTCCACGTTGACAAATCC	CCATCAATGAGACCCCAGAA	199
Am036	GAAA	NED-ATTCTTGCAATCCGTCGAGT	TGCACAGCTCCTGATCATCT	195
Am059	CAAA	VIC-GAATTCCATCAGTGGCCAAG	CTTCTGGGAAGACGCTGGT	241
Am088	GAAA	NED-TTGTTAGGCGTCAATCATGTG	ATCCTGCTGGTGTCGATCTT	211
Am092	CAC	6FAM-CCATGAGTTCTGGCGGTAAT	GCACGGTCACGATACTTATGAA	162
Am109	CAC	VIC-ATGAGACCCCAGAAGTTGAAGA	CACGTTGACAAATCCAATGC	188