Microbial- and Isothiocyanate-Mediated Control of *Phytophthora* and *Pythium* Species[1]

M.F. Cohen,[2] E. Yamamoto,[2] E. Condeso,[2] B.L. Anacker,[3] N. Rank,[2] and M. Mazzola[4]

Abstract

Plant pathogens of the oomycete lineage share common susceptibilities to many biotic and abiotic stresses. We are investigating the potential of antagonistic bacteria, isothiocyanates, and mycophagous amoebae to control diseases caused by *Phytophthora* spp., including the etiologic agent of sudden oak death, *Phytophthora ramorum* (Rizzo and others 2005), and *Pythium* spp., which cause seedling root rotting diseases across the plant kingdom (Hendrix and Campbell 1973).

Antagonistic bacteria. Inoculation of plant growth media with surfactant-producing bacteria is an established method for preventing oomycete diseases in hydroponic cultures. For instance, infection of water hyacinth by *Pythium ultimum* can be controlled by *Pseudomonas fluorescens* strain SS101, which releases a cyclic lipopeptide surfactant that lyses oomycete zoospores (deSouza and others 2003). We sought to determine whether *P. fluorescens* SS101 treatment could be effective in non-hydroponic systems by investigating its capacity to control root infection by soil-borne *Pythium* spp. and leaf infection by *P. ramorum*.

Cells of strain SS101 and the surfactant-deficient mutant strain 17.18 were inoculated into orchard soil, with concomitant addition of soy flour to stimulate amplification of resident *Pythium* spp. populations. Twelve wheat seeds were sown per treatment. After 24 days seedlings were harvested and 10 1 cm root segments per seedling assayed for infection by *Pythium* spp. Observed infection frequencies of 0.83 percent and 1.7 percent in the SS101- and 17.18 treatments, respectively, were both significantly lower ($P < 0.001$) than the 26.7 percent infection frequency in the nontreated control, implying that the surfactant is not necessary for disease control. These results are consistent with our recent findings of protection against *Pythium* spp. root infection conferred by another surfactant-deficient mutant of strain SS101.[5]

Although we observed that pretreatment with strain SS101 can dramatically lower the incidence *P. ramorum* zoospore-mediated infection of detached bay and rhododendron leaves, the pretreatment was not effective at reducing disease incidence in field trials, most likely due to the diminution of bacterial inoculum on the leaf surface resulting from precipitation events.

---

[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

[2] Department of Biology, Sonoma State University, 1801 E. Cotati Ave., Rohnert Park, CA 94928.
[3]Department of Geography and Global Studies, Sonoma State University, 1801 E. Cotati Ave., Rohnert Park, CA 94928.
[4]Tree Fruit Research Laboratory, USDA-Agricultural Research Service, 1104 N. Western Ave., Wenatchee, WA 98801.
Corresponding author: M. F. Cohen, cohenm@sonoma.edu.

[5] Mazzola, M.; Zhao, X.; Cohen, M.F.; Raaijmakers, J.M. 2007. Cyclic lipopeptide surfactant production by *Pseudomonas fluorescens* SS101 is not required for suppression of complex *Pythium* spp. populations. Phytopathology. 97: 97(10):1348–1355.

***Brassica* spp. seed meals.** Reapportionment of resident soil microbial populations following amendment with *Brassica napus* seed meals can convert a disease-conducive soil into one that suppresses fungal infection of new plantings (Cohen and others 2005; Mazzola and others 2001). The effect appears to result primarily from activation of plant systemic resistance and not direct antagonism of the fungal pathogens (Cohen and Mazzola 2006). If low glucosinolate content seed meal (<30 μmol g^{-1}) is utilized, a post amendment incubation period of several weeks is required in order to permit amplified *Pythium* spp. populations to decline in virulence potential (Cohen and Mazzola 2006). However, many *Brassica* spp. seed meals are high in glucosinolates that upon hydrolysis release isothiocyanates, some of which are highly toxic to oomycetes (Manici and others 1997). We sought to assess the ability of a high-glucosinolate *Brassica juncea* seed meal to inhibit amplification of *Pythium* spp. and, thus, obviate the need for a long post-amendment fallow period.

Seed meal of *B. juncea* cv. Pacific Gold contains high levels (303 μmol g^{-1}) of sinigrin, a glucosinolate that releases allyl-isothiocyanate upon contact with moisture. Incorporation of Pacific Gold seed meal into soil at 0.5 percent (vol/vol) resulted in dramatic long-term reductions in culturable oomycetes and prevented *Pythium* spp. infection of apple seedlings planted into the treated soil (Table 1), consistent with published results (Mazzola and others 2007).

Table 1—*Pythium* spp. population densities in soils and infection frequencies of Gala apple seedling roots

		CV soil		WVC-A soil	
Week[1]	Treatment	cfu/g DWsoil (x 10^2)	Percent Root infection[2]	cfu/g DWsoil (x 10^2)	Percent Root infection[2]
1	Control	0.38 ± 0.24[b]	7.5 ± 2.5[b]	0.80 ± 0.16[a]	11.0 ± 4.8[b]
	Seed meal	n.d.[a]	0.5 ± 0.5[b]	0.24 ± 0.15[a]	n.d.[a]
4	Control	4.0 ± 0.3[c]	27.5 ± 5.3[c]	0.54 ± 0.15[a]	40.5 ± 5.7[c]
	Seed meal	0.44 ± 0.33[b]	n.d.[a]	n.d.[b]	n.d.[a]

[1]Time after incorporation of 0.5 percent (vol/vol) *B. juncea* var. Pacific Gold seed meal amendment into soil.
[2]Root infection frequency values (means ± SE) within a column followed by the same letter are not significantly different. n.d., not detectable.

To determine the ability of *B. juncea* Pacific Gold seed meal to suppress *Capsicum annum* (Bell pepper) seed infection by *Phytophthora capsici*, 3-L samples of garden soil were subjected to various treatments and divided into duplicate plots that were each planted with 25 seeds. Germination frequency after four weeks was 68 percent in unmodified garden soil compared to 6.7 percent in the same soil infested with *P. capsici*. Amendment of *P. capsici*-infested soil with Pacific Gold seed meal immediately prior to planting resulted in a higher germination frequency (46 percent) than did amendment with low-glucosinolate content *B. napus* seed meal (10 percent), or ground *Azolla filiculoides* (12 percent), which does not contain glucosinolates.

Amoebae. Mycophagous amoebae, which consume both fungi and oomycetes, are thought to contribute to the disease suppressiveness of some soils. Amoebae are known to exist on leaves but their role in the phyllosphere habitat is not well characterized (Rodriquez-Zaragoza 1994). From *P. ramorum*-infected leaf lesions of California bay laurel (*Umbellaria californica*), we have isolated several strains of amoebae that are capable of subsisting on cultured *P. ramorum*, one of which, ANN04-395, displays apparent feeding on sporangia (fig. 1). Strain ANN04-395 has been separated from the original host by culture on heat-killed bacteria

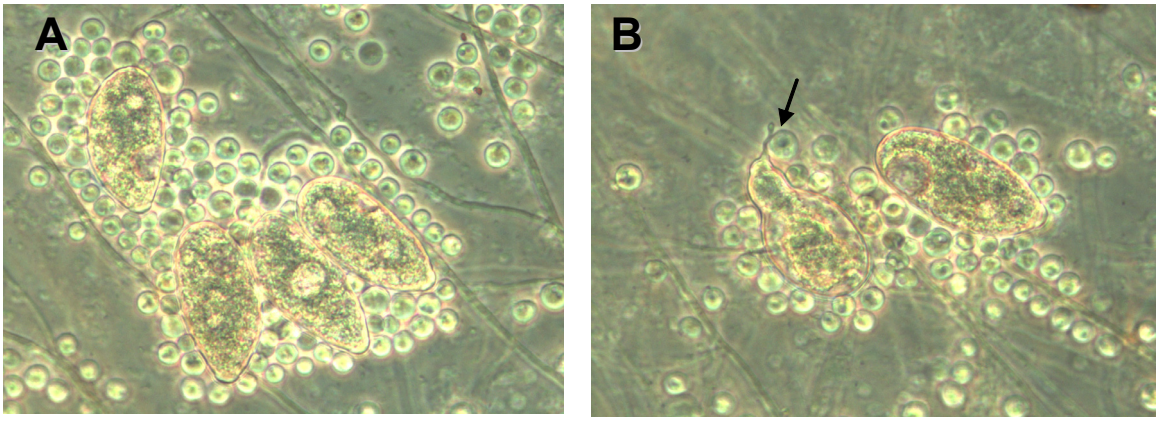

Figure 1—Amoeba strain ANN04-395 associated with *Phytophthora ramorum*. Trophozoites and cysts clustered around sporangia and hyphae of *P. ramorum* (A). Putative feeding of a trophozoite (arrow) on an emergent hypha of an apparently aborted sporangium (B).

and subsequently re-established on another strain of *P. ramorum*. Mycophagy by strain ANN04-395 is not specific to *P. ramorum*, as it is also able to feed upon *P. capsici* and an unidentified ascomycete. To assess biocontrol potential, bay leaves will be pre-treated with amoeba suspensions and assayed for susceptibility to *P. ramorum* and production of functional sporangia within infected lesions. Further development of biologically-based treatments may prove valuable for eliminating *P. ramorum* infestations in nurseries and ameliorating disease severity in landscapes.

Key words: Oomycetes, *Brassica* seed meal, mycophagous amoeba, *Pseudomonas fluorescens.*

Acknowledgments

Funding for this project has been provided in part from grants to M. Cohen and R. Meentemeyer from the United States Department of Agriculture (USDA)-Forest Service, Pacific Southwest Research Station Sudden Oak Death Research Program and to M. Mazzola from the USDA-CSREES Organic and Integrated Grants Program. We thank Jos Raaijmakers of Wageningen University for providing strains SS101 and 17.18 and Jim Prince of California State University, Fresno for providing *P. capsici*.

Literature Cited

Cohen, M.F.; Mazzola, M. 2006. Resident bacteria, nitric oxide emission and particle size modulate the effect of *Brassica napus* seed meal on disease incited by *Rhizoctonia solani* and *Pythium* spp. Plant and Soil. 286: 75–86.

Cohen, M.F.; Yamasaki H.; Mazzola, M. 2005. Modification of microbial community structure, nitric oxide production and incidence of Rhizoctonia root rot in response to *Brassica napus* seed meal soil amendment. Soil Biology and Biochemistry. 37(7): 1215–1227.

de Souza, J.T.; de Boer, M.; de Waard, P.; van Beek, T.A.; Raaijmakers, J.M. 2003. Biochemical, genetic, and zoosporicidal properties of cyclic lipopeptide surfactants produced by *Pseudomonas fluorescens*. Applied Environmental Microbiology. 69(12): 7161–7172.

Hendrix, F.F.; Campbell, W.A. 1973. Pythiums as plant pathogens. Annual Review of Phytopathology. 11: 77–98.

Manici, L.M.; Lazzeri, L.; Palmieri, S.J. 1997. *In vitro* fungitoxic activity of some glucosinolates and their enzyme-derived products toward plant pathogenic fungi. Journal of Agricultural Food Chemistry. 45: 2768–2773.

Mazzola, M.; Brown, J.; Izzo, A.D.; Cohen, M.F. 2007. Mechanism of action and efficacy of seed meal-induced pathogen suppression differ in a Brassicaceae species and time-dependent manner. Phytopathology. 97(4): 454–460.

Mazzola, M.; Granatstein, D.M.; Elfving, D.C.; Mullinix, K. 2001. Suppression of specific apple root pathogens by *Brassica napus* seed meal amendment regardless of glucosinolate content. Phytopathology. 91(7): 673–679.

Rizzo, D.M.; Garbelotto, M.; Hansen, E.M. 2005. *Phytophthora ramorum*: integrative research and management of an emerging pathogen in California and Oregon forests. Annual Review of Phytopathology. 43(1): 309–335.

Rodriquez-Zaragoza, S. 1994. Ecology of free-living amoebae. Critical Reviews in Microbiology. 20(3): 225–241.