Biological relevance of siRNA binding by the tombusvirus P19suppressor of silencing

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The p19 protein (P19) encoded by Tomato bushy stunt virus (TBSV) is an important contributor to pathogenesis and an effective suppressor of RNAi, which is thought to be related to its capacity for binding silencing-associated siRNAs. To examine the correlation between pathogenicity, RNAi, and siRNA binding, we performed bioassays with an infectious TBSV mutant expressing P19/75-78 with two R to G substitutions. TBSV expressing this mutant protein has a movement and symptom phenotype comparable to that observed for a mutant devoid of P19 expression. Northern blot assays with viral RNA from infected plants show that P19/75-78 is unable to prevent the RNAi-mediated degradation of TBSV RNA. Gel-filtration chromatography and immunoprecipitation experiments demonstrate that P19/75-78 retains the capacity to form dimers in plants, but the substitutions abolished siRNA binding, thus providing a biochemical explanation for its inability to suppress RNAi. Infection with another TBSV P19 mutant (P19/K60A) resulted in an intermediate phenotype compared to wild-type and P19/75-78, and its effect on RNAi and siRNA binding is being studied. Our hypothesis is that subtle structural effects in P19/75-78 compromise siRNA binding whereas the mutation in P19/60 affects a specific siRNA-binding contact site.

Mefenoxam and propamocarb sensitivity of *Phytophthora cinnamomi* at ornamental plant nurseries in Virginia

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Phytophthora cinnamomi is an aggressive pathogen of a wide range of woody ornamental plants. The current approach to control of root rots caused by this pathogen includes fungicides. The aim of this study was to detect fungicide resistance in *Phytophthora cinnamomi* to mefenoxam and propamocarb, two commonly-used fungicides. Sixty-four isolates were assessed using clarified V8 juice agar amended with mefenoxam at 100 μ g/ml. Eight isolates grew 10% to 18% as compared to growth on non-amended controls. Over 90% of the isolates grew 80% relative growth or greater on 1.8 mg propamocarb/ml amended agar as compared to growth on non-amended agar, indicating that propamocarb resistance is common. Ten isolates were selected for further assessment of the effective concentration (EC50) of these two compounds. This was accomplished using clarified V8 agar amended with 0, 0.1, 1, 10, 50, 100, 500, and 1000 μ g/ml of mefenoxam or 0, 1, 10, 25, 50 and 100 mg/ml of propamocarb. The mefenoxam EC50 ranged from 0.03and 0.12 μ g/ml and from 1.9 to 7.9 mg/ml for propamocarb.

High soil microbial diversity correlates with pathogen invasion but suppresses pathogen activity

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Soil contains the largest diversity of all terrestrial ecosystems, which has been suggested to enhance soil stability through reducing the growth and activity of plant pathogens. However, this assumption has not been vigorously tested and direct evidence illustrating diversity-led resistance to pathogen invasion and subsequent stability is still lacking. We examined the relationship between soil microbial diversity and invasion by the soilborne pathogen Pythium ultimum using diversity gradients in natural soils as well as in reconstructed microbial communities. The reconstructed microbial diversity gradient was created through reciprocally introducing microbial communities from three soils with significantly different microbial activities and diversity. Results obtained indicated that invasion and population density of P. ultimum was predominantly determined by soil resource availability (mainly available C and N). However, high microbial diversity significantly suppresses the activity of soilborne pathogens, thereby reducing seedling mortality of host plants. These results demonstrate that microbial diversity differentially impacts pathogen populations and their activities, which may represent an important mechanism through which high diversity enhances ecosystem stability.

Functional analysis of Meloidogyne incognita parasitism genes

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Parasitism genes expressed in the esophageal gland cells of Meloidogyne incognita encode secretory proteins that dramatically manipulate host-root gene expression during parasitism. Over 50 M. incognita parasitism gene candidates with 82% pioneers (unknown function) have been identified by directly analyzing the transcriptome of the nematode's esophageal gland cells. Polyclonal antisera raised against seven of the parasitism genes have been generated to immunolocalize parasitism gene products within the nematode, stylet secretions and nematode infected root tissues. An integrated approach of functional analyses consisting of over-expression of the parasitism genes in tobacco hairy roots and Arabidopsis, yeast-two-hybrid screen for identifying interacting host proteins, and gene silencing via RNA interference technology has been used to determine the roles of parasitism genes in the nematode-host interaction. Functional analysis of M. incognita parasitism genes is increasing our understanding of root-knot nematode parasitism of plants and is providing a framework for developing new biotechnological strategies for managing these nematodes.

Association of severe *Corynespora* root rot of soybean with glyphosatekilled ragweed

Presenter: D. Huber, Purdue University

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The soilborne pathogen Corynespora cassiicola was the predominant fungus isolated from severely stunted soybeans adjacent to glyphosate-killed giant ragweed plants (Ambrosia trifida) in Indiana fields. Soybeans adjacent to glyphosate-killed ragweed exhibited dark-brown to black lesions on 90-95% of their roots and hypocotyls. In contrast, soybeans that were not adjacent to dead Ambrosia trifida, or that were adjacent to living ragweed plants, exhibited only 5-10% root rot; and a number of different soilborne fungi in addition to Corynespora were isolated from these roots. Dead ragweed roots generally yielded pure cultures of Rhizoctonia and were not colonized by Corynespora. Koch's postulates were completed in the greenhouse where typical hypocotyl lesions developed in 3-5 days and lateral, "fine feeder roots" were extensively rotted by Corynespora. Soybean yield reduction was related to the density of glyphosate-killed ragweed plants and ranged from 1.5 kg per dead ragweed to 6 kg per dead ragweed in replicated field plots with and without killed ragweed plants. These field observations indicate that glyphosate or metabolites in dying ragweed root exudates modify the soil environment to predispose adjacent glyphosate-resistant soybean roots to severe *Corynespora* root rot even at temperatures above 20°C.

Brome mosaic virus in Alabama

Presenter: R. Huettel, Auburn University Phytopathology 95:S45

Brome mosaic virus (BMV) was first described in Alabama in 2002 from wheat samples in Henry County. Since then BMV has been found in all wheat growing areas thus far sampled with a total of 8 counties testing positive for the virus. Surveys for the virus were conducted by collecting 150 wheat leaves per field sampled. The collected wheat leaves were tested for the presence of BMV using direct double antibody sandwich ELISA kit (Agdia, Inc). The Alabama isolate of the virus is being compared to a known isolate from Oklahoma. This BMV virus may be of importance to wheat growing areas of the southern states where wheat is an increasing important winter cover crop used for both seed and feed.

Effect of bird cherry-oat (BCO) aphids and barley yellow dwarf virus (BYDV) on winter wheat

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Winter wheat frequently is planted in September in Oklahoma, and then used as forage for cattle during the fall and winter. However, this early-planted wheat often is infested with BCO aphids (*Rhopalosiphum padi*), which can affect the wheat either by feeding or by transmitting BYDV. Hence, this study was conducted to attempt to determine the impact of aviruliferous (AVR) and