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Fungicide resistance of *Botrytis cinerea* from Virginia grapes, strawberries, and ornamentals

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Botrytis cinerea causes gray mold on grapes and diseases of other host such as fruits, vegetables, and ornamental crops. It has developed resistance to many fungicides, and isolates from eastern U.S. strawberry fields have been identified with resistance to several modes of action. During the 2011-2015 growing seasons, 487 isolates were collected from Virginia grapes, strawberries, and ornamental crops and evaluated for sensitivity to eight different fungicides by a germ tube elongation method; for a subset of isolates, a 24-well mycelial growth protocol (Fernández-Ortuño et al. 2014. *Plant Dis.* 98:825) was also used, and baseline sensitivity to polyoxin-D was evaluated. High resistance to benzimidazoles and QoI, and low-level resistance to iprodione were common. High boscalid resistance was common in grapes and ornamentals. Fenhexamid resistance was found in only 5% of grape isolates, but in 33% of isolates from strawberries and ornamentals. None of the fenhexamid-resistant isolates were identified as *B. pseudocinerea*, a cryptic species reported in other countries which is naturally resistant to fenhexamid; all were *B. cinerea* carrying mutations in the Erg27 gene. Moderate resistance to cyprodinil was common, but in grape inoculation tests, moderately resistant isolates were controlled by a field rate of cyprodinil. Diminished sensitivity to fludioxonil and fluopyram was rare. Polyoxin-D controlled most isolates in mycelial growth tests at 100 ug/ml.

Managing oomycete populations at irrigation-associated critical control points in ornamental greenhouses

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The ornamental greenhouse industry must meet marketing demands for quality. To do so, ornamental growers are encouraged to implement an adequate disease management plan. This includes identification and management of critical control points (CCPs) regulating introduction and in-house spread through irrigation water. The objectives of this study were to evaluate water-based (CCPs) for poinsettia root rot pathogen management, and test the use of deficit irrigation strategies as a way to mitigate water-based CCPs. Analysis of pond water irrigation as a source of oomycete pathogens (based on 123 symptomatic rhododendron bait leaves) identified 113, seven and zero oomycete from the pond, pump house, and well water baits respectively, and included *Phytophthora litorale* (64%), *Phytophthora hydropathica* (11%) and *Phytophthora cryptogea* (11%). Pathogens contributing to in-house spread were evaluated in poinsettias, because production is severely impacted by oomycete pathogens. Analysis of 53 symptomatic poinsettia revealed two dominant oomycete pathogens: *Pythium aphanidermatum* (81% of isolates), *P. adhaerens* (11% of isolates); as neither species is reported as pathogen of poinsettia in Maryland, pathogenicity trials are underway. Preliminary deficit irrigation studies indicate a higher root rot incidence in the 48% WVC deficit irrigation treatment compared to the saturated (60% VWC) irrigation treatment but differences were not significant. Cumulatively, these studies indicate that *Pythium* spp. are the primary oomycete pathogens to target for control of poinsettia root diseases, and pond water is a potential source of oomycete pathogens, which may be controllable with deficit irrigation technologies.

Control of anthracnose (caused by *Colletotrichum gloeosporioides* s.l.) on mango in Senegal by fungicides and biofungicides

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Senegal ranks second for mango production in West Africa, but anthracnose can be a serious problem in some regions and/or years, causing premature fruit drop and pre- and post-harvest fruit rot. *Colletotrichum gloeosporioides* Penz and Sacc. has historically been named as the causal agent, but is currently considered a species complex. Field trials to examine the efficacy of fungicides and biofungicides were conducted in three orchards in southern Senegal. Overall, Sonata (*Bacillus pumilus* strain QST 2808), Serenade Optimum (*Bacillus subtilis* strain QST 713), and sodium molybdate (90 g/ha) provided 63%, 67% and 76% control of disease severity respectively, whereas thiophanate methyl and azoxystrobin provided 77% and 78% control, respectively. Treatment effect on disease severity at all three locations was significant ($P < 0.05$). All treatments resulted in lower disease severity than the control, and thiophanate methyl and azoxystrobin resulted in lower disease severity than other treatments at all three locations. Numerically, thiophanate methyl and azoxystrobin produced more disease-free mangoes (64 and 62%, respectively) than Serenade (49%), sodium molybdate (44%), and Sonata (38%). Sequencing the ITS gene region of 30 *Colletotrichum* isolates from mangoes from different regions in Senegal showed that all isolates belong to the *C. gloeosporioides* species complex. To further characterize isolates, sequencing of loci within the *Apn2/Mat* region is in progress.

Fungicide carrier volume affects curative applications for dollar spot disease of creeping bentgrass

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In turfgrasses, disease management often entails multiple costs. In some instances, fungicide carrier volume can be adjusted to cover larger areas, thereby cutting fuel and time costs. Previous research has shown that carrier volume impacts fungicide efficacy, but newer products have not been tested. In 2015, a trial was developed to evaluate the impact of carrier volume on curative dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) applications of fungicides on a creeping bentgrass (*Agrostis stolonifera* cv. "007") putting green. Dollar spot developed naturally on 16 May. Fungicides [fluazinam (FZM), fluopyram (FPM), fluxapyroxad + pyraclostrobin (FLX+PYR), chlorothalonil (CHL), and propiconazole (PRO)] were applied at 815 L ha⁻¹ and 408 L ha⁻¹ using a CO₂ powered backpack sprayer with TeeJet AI9508E (815 L) or TeeJet AI9504E (408 L). Treatments were applied from 25 June to 4 August. Turf was evaluated for infection every 7-14 d. While fungicides reduced dollar spot, CHL and FPM provided the most rapid reduction in disease. Following the second treatment, FPM, FLX+PYR, and PRO applied in a 815 L ha⁻¹ carrier volume provided more control than 408 L ha⁻¹. In contrast FZM provided better control in 408 L ha⁻¹ during the same time period. After suspending treatments, FPM and FLX+PYR provided the longest residual control of dollar spot. Turf color and quality were both improved in fungicide treated plots when compared to the control, irrespective of spray volume.

Transcriptome differences between PRSV susceptible and resistant transgenic papaya cultivars

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Papaya Ringspot Virus (PRSV) is one of the most devastating papaya diseases worldwide. Development of transgenic resistant varieties is considered as the most effective strategy to control this disease. However, little is known about the genome-wide functional change induced by particle bombardment transformation. Thus, we conducted RNA-Seq of PRSV resistant transgenic papaya SunUp and its PRSV susceptible progenitor Sunset to compare the transcriptional changes in healthy leaves before they infected with PRSV. In total, 20,700 transcripts were identified and 842 differentially expressed genes (DEGs) randomly distributed amongst papaya chromosomes were found between these two cultivars. Gene ontology (GO) category analysis revealed that microtubule-related categories were highly enriched among these DEGs. On the basis of KEGG enrichment analysis, no significant pathways were detected with the corrected *p*-values smaller than 0.01. Numerous DEGs related to various transcription factors, transporters and hormone biosynthesis showed clear differences between the two cultivars. Many known and novel stress-induced and disease-resistance genes were most highly expressed in SunUp, such as *MYB*, *WRKY*, *ERF*, *NAC*, nitrate and zinc transporters, and genes involved in the abscisic acid, salicylic acid, and ethylene signaling pathways. We also identified 67,686 alternative splicing (AS) events in Sunset and 68,455 in SunUp, mapping to 10,994 and 10,995 papaya annotated genes, respectively. GO enrichment for the genes displaying AS events exclusively in Sunset was significantly different from that in SunUp. Our results indicated that transcriptomes in Sunset and SunUp shared a lot of similarities with a few noteworthy differences which are determinant factors to the increased resistance in transgenic papaya. This study facilitate a better understanding of the global impact of bombardment-mediated transformation on whole genome function in papaya plants, providing a valuable foundation for additional research on crop breeding and genetic engineering of papaya and related species.

Quinoxifen resistance frequency and effect on powdery mildew control in a resistance-affected vineyard

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Quinoxifen has been used against grape powdery mildew (*Erysiphe necator*) in the U.S. since 2003. In 2013, quinoxifen resistance in grape powdery mildew was detected in a single vineyard in Virginia. Field trials were conducted in 2014 and 2015 at the affected vineyard, to establish resistance frequency, and to determine to what extent quinoxifen might still contribute to disease control. In 2014, four treatments including myclobutanil+sulfur, quinoxifen+boscalid+myclobutanil+sulfur, quinoxifen and metrafenone were applied to Chambourcin plots at 2-week intervals. Surprisingly, quinoxifen treatment still provided good control. In 2015, a similar field trial was conducted on Chambourcin and Pinot Noir plots. Little disease developed in the Chambourcin. In Pinot Noir, quinoxifen provided good control on clusters rated on July 14. However, significantly more powdery mildew had developed in the quinoxifen plots than in the other treatments at a foliar rating a month later. When single-spored isolates from non-quinoxifen vines were bioassayed, the resistance frequency was 65% (n=124) in 2014, and 50% (n=224) in 2015, suggesting a slow decline in the absence of quinoxifen treatment. The average resistance frequency (2014 and 2015) in quinoxifen-treated plots (78%) was only modestly higher than that in nontreated plots (55%).

Field level risk assessment for root-knot nematodes in lima beans

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Southern Root-Knot Nematode (RKN), *Meloidogyne incognita*, is a major yield limiting pest in lima beans (*Phaseolus lunatus*). RKN are not evenly distributed through fields and population dynamics are fluid, making whole field management challenging. The objectives of this research were to characterize RKN distribution in fields in relation to soil factors and to quantify RKN populations and impacts on root infestation of lima beans in order to develop field-level risk assessments. Surveys were conducted on eight lima bean fields with a history of RKN in 2014 and six fields in 2015. Fields were divided into one hectare grids and evaluated for edaphic parameters (field water capacity (FC), electrical conductivity, depth to hardpan, and pH). Twenty soil cores were taken at a 30 cm depth within each grid for nematode assays. Fields were sampled in fall, spring, summer, and subsequent fall and spring. Plant growth was evaluated indirectly using NDVI. RKN second stage juveniles were extracted from soil samples using elutriation and sucrose centrifugation. Greenhouse cucumber seedling bioassays were conducted on grid soil samples and rated for galling on a 1 to 10 scale. Test crop roots were evaluated for gall ratings at mid-season and after-harvest. Spring J2 counts were significantly correlated with after-harvest root ratings ($p=.009$, $R^2=.431$); whereas bioassays were less predictive ($p<.001$, $R^2=.335$). Of the edaphic factors, FC was also correlated with after-harvest root ratings ($p<.001$, $R^2=.111$). A model combining spring J2 counts and FC gave best prediction of lima bean galling ($P<.001$, $R^2=.524$). This research will be used to develop site-specific RKN management practices for lima beans.

Evaluation of nematicides for control of Southern root-knot nematodes in lima bean

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Southern root-knot nematodes (*Meloidogyne incognita*-RKN) significantly reduce lima bean (*Phaseolus lunatus*) yields. Chemical control options for RKN are limited. We evaluated the efficacy of new nematicides on RKN in lima bean experiments conducted in greenhouse (GH) and microplot (MP) settings. Treatments included Nimitz at either low or high labeled rates, Luna, Movento, Luna+Movento, Vydate, Mocap and untreated, RKN infested controls. GH treatments were arranged in a factorial design, with application of nematode eggs (6,000 or 30,000 eggs pot⁻¹) crossed with nematicide treatment. MP treatments were arranged in a randomized complete block design and all plots were infested with 4.80 kg of RKN infected tomato root tissue and soil. Root galling, RKN eggs and juveniles, and aboveground plant dry masses were determined and statistically analyzed using Mixed Model

ANOVA. In the GH study, Nimitz at both rates provided the greatest reduction in RKN galling compared to the controls, whereas Movento treatments were not significantly different than the controls. In the MP study, all treatments except Movento significantly reduced RKN populations relative to controls. Aboveground plant dry masses were significantly greater for Vydate, Nimitz (low rate), Mocap, and Luna+Movento treatments compared to Nimitz (high), Luna, Movento, and controls in the MP study. Based on these results, several new nematocides show potential for managing RKN in lima beans.

Genetics of bacterial virulence in *Erwinia amylovora* and beyond

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Erwinia amylovora is the causal agent of fire blight, an economically important bacterial disease of Rosaceous plants. Several *E. amylovora* pathogenicity and virulence factors are already known, including the extracellular polysaccharide amylovoran and a type III secretion system, which delivers effector proteins into host cells. Our lab identifies novel virulence factors required for fire blight disease development by screening Tn5 transposon mutants for a loss of disease on immature apple fruit. This screen has identified 164 virulence defective mutants, representing 85 unique genes, including many genes not known to be involved in fire blight. One of these novel genes is *rfbX*, a putative polysaccharide transporter. To help describe the function of *rfbX*, we have identified several genes that, when mutated, restore virulence in an *rfbX* background. Additionally, we identified a gene mechanistically involved in protein elongation that we have demonstrated to be necessary for the accumulation of a large subset of proteins, including those related to virulence and basic cellular processes. This represents an example of using *E. amylovora* to explore fundamental biological activities. Furthermore, several genes identified in our screen have virulence homologues in related species such as enteropathogenic *E. coli* and *Salmonella* spp., raising the possibility that the fire blight pathosystem could be used to uncover virulence functions relevant to mammalian pathogenic systems.

Survival biology of *Botrytis cinerea* sclerotia in agricultural fields and the role of soil amendments in breaking the gray mold disease cycle

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Botrytis cinerea causes major pre-harvest losses of strawberries in the Mid-Atlantic. Management challenges have escalated since the loss of soil fumigants that restricted debris- and soil-borne inoculum buildup. In a summer 2015 field survey, Maryland strawberry fields had up to 350 *B. cinerea* cfus per gram of soil and 20% yield losses from gray mold. The objective of this project was to evaluate the efficacy of soil amendments in degrading *B. cinerea* sclerotia – the most resilient source of inoculum. In potted studies under controlled conditions, mixtures of leaf compost, manure, and crab meal were evaluated for effects on sclerotia decomposition compared to untreated soil. 60 days after burial, sclerotia viability decreased in the crab meal and leafgro treatments by 91.7% ($\pm 5\%$) and 96.7% respectively, compared to 76.7% in untreated soil. Bacterial and yeast colonization of sclerotia was highest in the crab meal treatment (91.7% $\pm 8.3\%$) and hyphal fungi colonization was highest in the leafgro treatment (93.8% $\pm 6.3\%$). Similar trends between sclerotia viability and colonization were seen in field trials on the Maryland Eastern Shore: 60 days after burial, the leafgro + crab meal treatment had the greatest sclerotia viability reduction and the highest bacterial/yeast colonization. These studies indicate that soil amendments may hold promise in reducing *B. cinerea* survival and dispersal in the Mid-Atlantic, in part by promoting microbial colonization of sclerotia.

Development of a collection of *Fusarium* fruit rot isolates from cantaloupe

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Cantaloupe (*Cucumis melo*) fruit are hosts to several fruit rots including those caused by *Fusarium* spp. As part of a study to evaluate whether *Fusarium* fruit rot plays a role in the ability of *Salmonella* to penetrate, colonize and multiply in cantaloupe fruit, a collection of *Fusarium* spp. from cantaloupe fruit was made. In 2015, cantaloupe fruit of the cultivar Sivan F1 were grown in an organically managed research field at the University of Maryland's Lower Eastern Shore Research and Education Center, Salisbury. Fruits showing visible fruit rot were collected over a period of one and half months. Eight mono-conidial *Fusarium* isolates were obtained, and tested for pathogenicity on cantaloupe by inoculating them onto fruit purchased from local retail stores. Fruit were either wounded or non-wounded prior to inoculation. In addition, the mono-conidial isolates were grown on quarter strength PDA and examined for the presence of microspores, conidial shape and other morphologically informative traits. DNA was extracted, amplified with PCR and sequenced for identification. A subset of these isolates will be used in co-inoculation studies to evaluate whether the presence of *Fusarium* fruit rot aids in the colonization of *Salmonella enterica* Newport.

The soybean cyst nematode, *Heterodera glycines*, female cyst is an environmental filter and a potential source of nematode control compounds

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The success of plant-parasitic nematodes as crop pathogens is evidenced by the parasites' significant and continuous economic drain on global agriculture. Despite continued efforts to control these pests, employing strategies from the environmental to molecular levels, annual economic losses have not decreased significantly over the last quarter century. A thorough understanding of the fundamental interactions of the nematode with its environment, throughout the life cycle, will significantly improve control agent development. Among the most economically important groups of plant-parasitic nematodes are the cyst-forming species, including the soybean cyst nematode *Heterodera glycines*. The *H. glycines* life cycle presents remarkable changes in behavior, morphology and developmental response to the environment. Prominent within the life cycle are the female cyst, its role in embryogenesis, dormancy and hatching, and its presence in the rhizosphere. We provide evidence that the *H. glycines* cyst influences embryonic development in response to the environment, and elucidate an extensive protease array within the cyst. Results suggesting that quantitative and qualitative differences in this array may be related to the environment as well as to developmental age are presented. We discuss the discovery of protease inhibitors within the cyst, identify the affected proteases, and present evidence that the effects of such inhibitors are species specific. Based upon biochemical and developmental evidence, the female nematode cyst represents a critical target that should be examined for fundamental insight into development and for novel nematode control compounds.

Redesignation of *Phytophthora* taxon walnut as *P. hindsii* sp. nov.

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Isolates of a new *Phytophthora* species, provisionally named as *P. taxon* walnut, were frequently recovered from pistachio orchards in Iran and irrigation water in Virginia, U.S.A. This species produced non-papillate and non-caducous sporangia. All examined isolates were compatibility type A1. They produced oogonia with amphigynous antheridia and plerotic oospores. Sequence of the rDNA internal transcribed spacer region and the mitochondrially encoded cytochrome *c* oxidase 1 genes placed this species in a distinct taxon within *Phytophthora* subclade 6a. Based on these unique morphological and

molecular features, *P. taxon* walnut is formally named as *P. hindsii* sp. nov. After its first host plant, *Juglans hindsii*. Formal description of this species will facilitate rapid identification of this pathogen and development of disease management strategies.

Genetic diversity of *Phytophthora parsiana* causing pistachio gummosis in Iran

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Phytophthora parsiana has been considered as a major pathogen of pistachio, causing crown rot and gummosis in Iran, the world's largest producer of this nut crop. Objective of this study was to determine the genetic diversity of *Phytophthora* isolates collected from pistachio over the past 25 years by sequencing the internal transcribed spacers (ITS) region, β -tubulin (β -tub), and mitochondrial cytochrome-*c* oxidase subunit 1 (*cox1*) genes. Among the three genetic markers, *cox1* was the most informative, followed by ITS, while β -tub sequences had numerous ambiguity reads. Phylogenetic analysis of *cox1* sequences indicated that the pistachio isolates formed three subgroups (taxa A, B, and D), all distinct from the type culture of *P. parsiana* (taxon C) that originated from fig. Similar clustering was observed in the analysis based on ITS sequences. These results indicate that there is tremendous genetic diversity in the culture collection previously identified as *P. parsiana*. Extensive polymorphisms in the ITS and β -tub sequences indicated the three subgroups of pistachio isolates examined in this study are hybrids. This study provides additional evidence that hybridization is an important mechanism for emerging plant pathogens.

Characterization and pathogenicity of black root rot of strawberry pathogens in the Mid Atlantic U.S.A.

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Black root rots (BRRs), considered to be among the most serious diseases of strawberry in the U.S.A., can be caused by diverse fungi, oomycetes, and nematode species, and may in some cases develop as disease complexes. In the mid-Atlantic region, BRR is a persistent problem, causing stunting, reduced yields, and plant death, but the causal agents are not known, hindering effective management. In 2015 field surveys to better understand BRR etiology, *Fusarium* spp. *Pythium* spp. and *Rhizoctonia* spp. were the most commonly recovered genera (isolated at frequencies 27.9%, 10.3% and 25.5% from plants respectively) across five farms. These genera co-occurred at four of the five farms, on 16-67% of plants, in which *Fusarium-Rhizoctonia* co-occurrence was the most common. Sequence-based analysis identified several species reported as BRR pathogens, including *Pythium irregulare*, *Rhizoctonia solani*, and *Fusarium oxysporum*. Trials are underway to evaluate pathogenicity of these species on diverse cultivars with reported BRR susceptibility. Downstream studies will evaluate the association of nematodes with BRR symptoms. Results of this study will identify the pathogens driving BRR in the mid-Atlantic, examine pathogen interactions, and evaluate BRR susceptibility and resistance traits.

Analysis of competitive transmission between JP430b and B239 strains of *Candidatus Liberibacter asiaticus*

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Huanglangbing (HLB, citrus greening) is considered the most destructive citrus disease that threatens U.S. agriculture. JP430b is a strain of *Ca. Liberibacter asiaticus* (Las) from Japan that has mild symptoms and low titer infections. B239 is a strain of Las from Taiwan that causes typical symptoms of HLB infection with normal titers. Las transmissions are typically more successful in cases of high titer symptomatic infection. The objective is to determine the relative competitive fitness of JP430b and B239. Healthy psyllids were fed on a JP430b infected plant and tested for the presence of Las. Half the psyllids were transferred to a plant infected with B239, and then on to healthy sweet orange seedlings. The other half of the psyllids were transferred directly to healthy sweet orange as a positive control for transmission. The reciprocal transmissions were done with psyllids first being fed on B239, then transferred to JP430b. Psyllids were able to acquire both strains of Las, and in single feed experiments were able to transmit the disease to healthy seedlings. However, when the psyllids were fed sequentially on one strain after the other, regardless of the order, only B239 was found present in the newly inoculated plants. This suggests that B239 is a more competitive Las strain. A real-time quantitative assay has been developed for more accurate analysis of JP430b titers in competitive experiments.

Germination of oospores of *Plasmopara obducens* and characterization of its megasporangia

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Megasporangia of *Plasmopara obducens*, the causal agent of Impatiens downy mildew, were described for the first time. Oospores of *P. obducens* were grown in large numbers in detached stem culture and induced to germinate by exposure to 0°C for at least a month. On three occasions when plant cortical tissue containing germinated oospores was placed on detached leaves of *I. balsamina*, downy mildew appeared on the leaves, fulfilling Koch's postulates for oospores. The formation of megasporangia from cold-treated oospores and their ability to infect *Impatiens* suggests that overwintering of the pathogen in colder regions as oospores is possible.

Evidence points to homothally in the *Impatiens* downy mildew

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To better understand the life cycle of the *Impatiens* downy mildew pathogen, *Plasmopara obducens*, it was useful to determine whether the organism was homothallic (self-fertile) or heterothallic (requiring two mating types to form viable oospores). To test for heterothally, isolates were produced from single sporangia and single zoospores, in an attempt to isolate individual mating types if they were present. Stem pieces of *Impatiens balsamina* were inoculated with single isolates or dual combinations of isolates and no differences in numbers of oospores forming or appearance of oospores were observed. Monocliny (fertilization of oögonia by antheridia on the same hypha) was commonly observed. Oospores produced from single-isolate infections of stem pieces and from dual inoculations germinated in both cases to produce megasporangia. If *P. obducens* is homothallic, as this evidence suggests, oospore formation may be common in the landscape, and the organism may be able to overwinter in infested beds.