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## **Endophytic *Bacillus* Species Confer Increased Resistance in Cotton Against Damping off Disease Caused by *Rhizoctonia solani***

L. Rajendran and R. Samiyappan

Endophytic bacterial strains were evaluated for their efficacy against the damping off disease caused by *Rhizoctonia solani* in cotton. Among hundred and three endophytic bacterial strains isolated, two strains (*Bacillus* sp. strains EPCO102 and EPCO16) significantly increased plant growth and inhibited the mycelial growth of *Rhizoctonia solani* *in vitro* conditions. The efficacy of talc-based bioformulation of endophytic bacterial strains, EPCO102, EPCO16 and *Pseudomonas fluorescens* strain Pf1 amended with and without chitin in inducing systemic resistance was tested against damping off disease under greenhouse conditions. The application of the bioformulation through seed, soil and foliar spray significantly reduced disease incidence under greenhouse conditions. The amendment of chitin in the formulation further reduced the disease incidence. EPCO102, EPCO16 and Pf1 strain along with chitin treatment was recorded 46.7, 53.3 and 40.0% damping-off incidence compared to control 83.3%. Treatment with the endophytic bacterial bioformulation increased the levels of the defence-related enzymes chitinase,  $\beta$ -1,3-glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase and phenol in cotton plants which had been challenged with *R. solani*. In addition to plant growth and antibiosis, endophytic bacterial strains enhanced the resistance in plants through the induction of defense enzymes in cotton plants. (*Plant Pathology Journal* 7 (1): 1-12, 2008; doi: 10.3923/ppj.2008.1.12)

## **Management of *Fusarium* Head Blight of Wheat and Deoxynivalenol Accumulation Using Antagonistic Microorganisms**

G.M. Riungu, J.W. Muthomi, R.D. Narla, J.M. Wagacha and J.K. Gathumbi

Laboratory and green house studies were conducted at the Faculty of Agriculture, University of Nairobi, to evaluate the efficacy of *Epicoccum* sp., *Alternaria* sp., *Trichoderma* sp. and *Bacillus* sp. in control of *Fusarium* head blight of wheat caused by *F. graminearum*. Fungicides folicur® and copper oxychloride were used as standard checks. Laboratory assay was carried out by paired cultures and antagonism was measured as reduction in pathogen colony diameter. Green house

experiments involved dual inoculation of pathogen and antagonist onto wheat ears and head blight severity and grain yield determined. Doxynivalenol content in the resulting grain was determined by competitive direct ELISA. The antagonists and fungicides significantly reduced the growth of *Fusarium graminearum* colonies in culture. Folicur® and copper oxychloride completely inhibited the growth of the pathogen while *Trichoderma* sp. showed 64% colony growth reduction. However, the antagonists showed limited reduction in head blight severity in green house trials. *Trichoderma* sp. reduced head blight severity by 18% while folicur® reduced the disease by 28%. All the antagonists had little or no significant effect on grain yield. Only folicur®, copper oxychloride and *Alternaria* sp. reduced DON in grain by 76 to 93%. Obtained results indicate that microbial antagonists may offer potential benefit in FHB management and screening of more antagonists both under controlled and field conditions is necessary. (*Plant Pathology Journal* 7 (1): 13-19, 2008; doi: 10.3923/ppj.2008.13.19)

### **Molecular Diversity of *Ascochyta rabiei* Isolates from Chickpea in Alberta, Canada**

K.F. Chang, S.F. Hwang, A.H. Khadhair, H.U. Ahmed, S.E. Strelkov, M. Deyholos, G.D. Turnbull and J. Feng

During the summer of 2003 and 2004, a total of 58 isolates of *Ascochyta rabiei* were collected from chickpea plants grown in southern Alberta, Canada. RAPD analysis of genomic DNA extracted from these isolates was conducted using six short sequence primers (OPA-03, OPA-13, OPB-07, OPC-01, OPC-20 and OPJ-15) and analyzed to establish the genetic relationship and distance between isolates. A total of 75 bands were polymorphic. The 58 isolates were found to belong to five genotypes, indicating that the *A. rabiei* population of southern Alberta is genetically diverse. No relationship was found between the genotype groupings obtained through RAPD analysis and previously determined pathotype classifications of the same isolates. (*Plant Pathology Journal* 7 (1): 20-26, 2008; doi: 10.3923/ppj.2008.20.26)

### **Effect of Environmental Conditions on Wilting and Root Rot Fungi Pathogenic to Solanaceous Plants**

El-Sayed A. Fayzalla, Yasser M. Shabana and Nasser S. Mahmoud

Twenty three isolates of *Fusarium oxysporum*, eight isolates of *Fusarium solani*, two isolates of *Verticillium dahliae* and four isolates of *Rhizoctonia solani* were

isolated from tomato plants showing wilting and root rot symptoms at different localities in Dakahlia governorate, Egypt. These isolates varied in their aggressiveness against tomato plants. The influence of temperature, pH, light regime, sealing culture plates with Parafilm (1-10 layers) and type of media on the growth of two *F. oxysporum* f. sp. *lycopersici* (isolates 14 and 19), *F. solani*, *V. dahliae* and *R. solani* were evaluated under laboratory conditions in Petri dishes or in liquid culture. The incubation conditions of 25°C and improved aeration (obtained by not wrapping the culture plates) induced the optimal growth of all fungi tested. Among the culture media tested, potato dextrose agar (PDA) was the best medium for the growth of all fungi tested except for *F. oxysporum* f. sp. *lycopersici* (isolate 14) which grew best on lima bean agar. The continuous light induced the best growth for *F. oxysporum* f. sp. *lycopersici* (isolate 19), *F. solani* and *R. solani*. However, *F. oxysporum* f. sp. *lycopersici* (isolate 14) grew best under continuous darkness while diurnal light was the best for *V. dahliae* growth. In general, pH 8 (initial level) promoted the best growth of all fungi tested (isolate 19 of *F. oxysporum* f. sp. *lycopersici*, *F. solani*, *V. dahliae* and *R. solani*) except for *F. oxysporum* f. sp. *lycopersici* (isolate 14) which was best grown at pH 9 (initial level). (*Plant Pathology Journal* 7 (1): 27-33, 2008; doi: 10.3923/ppj.2008.27.33)

### **Genotypic Variability for *Alternaria alternata* (Fr.) Keissler Infection in Bread Wheat**

Muhammad Shahab Saqib, S. Jawad A. Shah, Fida Muhammad, Muhammad Ibrahim and Sajid Ali

Naturally infected grains of nineteen candidate wheat lines along with local check were studied for black point symptoms at Nuclear Institute for Food and Agriculture (NIFA), Peshawar, during 2005-06. Data were recorded on the percent incidence, black point symptoms and thousand grain weights. Results indicated that all tested candidate varieties were found susceptible to *A. alternata* but they differed in symptoms, incidence and severity of infection. Variability in number of affected kernels among candidate varieties fluctuated between 1.2-20.1% while its mean was <10%. Low level incidence (i.e., <3%) was recorded in three candidate varieties i.e., 99B4012, NRDW-1 and Wafaq 2001. Percentage of the kernels with >50% disease coverage (i.e. infection severity) varied among the tested cultivars and it was highest (i.e., 30%) in V-99022. Symptoms which were observed in all cultivars included black point, dark smudge and black specks. Based on the disease incidence level of each candidate variety, an inference regarding disease situation under field conditions was derived in which

up to 46 infected seeds capable of producing disease will be entering per m<sup>2</sup> of the field. Field prediction along with incidence and symptoms severity may be utilized as selection criteria for commercial and upcoming breeding lines and cultivars. (*Plant Pathology Journal* 7 (1): 34-39, 2008; doi: 10.3923/ppj.2008.34.39)

### **Sources of Tolerance to Root-Knot Nematode, *Meloidogyne javanica*, in Cultivated and Wild Tomato Species**

Idorenyin A. Udo, Michael I. Uguru, Rufus O. Ogbuji and Donald A. Ukeh

Five elite tomato cultivars (HT-5044, HTA-18, HTA 19, HTA-31 and HT-Ravid 244), two locally grown Nigerian cultivars (Roma VF and Nsukka local) and a wild tomato related species (*Lycopersicon pimpinellifolium*) were assayed for their responses to an indigenous population of *M. javanica* in the greenhouse. Infected plants were scored for galling on a 0-5 rating scale. The cultivars HTA-18 and HTA-31 had significantly ( $p < 0.05$ ) fewer gall counts per root system and per gram fresh weight of root than the other cultivars. Single degree of freedom analysis showed that HTA-18, HTA-19 and HTA-31 had significantly ( $p < 0.05$ ) fewer galls than the locally grown cultivars. HTA-18 and HTA-31 were moderately susceptible with Gall Index (GI) of 3, while the rest of the cultivars were highly susceptible ( $GI > 4$ ). The nematode caused a significant delay in flowering and a decrease in fresh and dry weights of roots, stems and leaves in the cultivars, HT-5044, Roma VF and Nsukka local. HTA-18, HT-Ravid 244 and the wild species were considered tolerant. (*Plant Pathology Journal* 7 (1): 40-44, 2008; doi: 10.3923/ppj.2008.40.44)

### **Pathogenicity Study and Nematotoxic Properties of Some Plant Extracts on the Root-Knot Nematode Pest of Tomato, *Lycopersicon esculentum* (L.) Mill**

T.I. Olabiyi

In the screen house, tomato seedlings, cv. DT 69/257, grown in steam-sterilized soil were inoculated with graded inocula of 5,000; 10,000; 15,000; 20,000 and 25,000 eggs of root-knot nematode, *Meloidogyne incognita*. At inocula levels of 15,000; 20,000 and 25,000 eggs of *M. incognita*, number of leaf per plant, plant height, fruit yield and root galls were significantly reduced. In the field planted with tomato seedlings, aqueous extracts from the roots of marigold, nitta and basil plant were applied to root-knot nematode inoculated soil at four levels of 25,000; 500,000; 750,000 and 1,000,000 ppm concentrations, 10 mL per tomato stand.

All the aqueous plant root extracts applied in the trials reduced root-knot nematode populations in the soil with corresponding increases in plant height, plant leaf and fruit yield over the control treatment. Significant reduction of root galls from the treated plots indicated effective root-knot nematode control by the aqueous root extracts. (*Plant Pathology Journal* 7 (1): 45-49, 2008; doi: 10.3923/ppj.2008.45.49)

### **Post-Harvest Applications of Phosphorous Acid Materials for Control of *Phytophthora infestans* and *Phytophthora erythroseptica* on Potatoes**

Steven B. Johnson

The purpose of this study was to determine the value of select phosphorous acid materials compared to existing post-harvest materials in preventing tuber-to-tuber spread of *Phytophthora infestans* and *Phytophthora erythroseptica* during the mechanical harvesting and tuber transfer in a situation where the diseases are present at harvest. Products evaluated over two seasons were Phostrol, Rampart and ProPhyt; these were tested against Oxidate and Agclor 310 for control of late blight and pink rot. Tubers were treated with the control materials one or three hours post inoculation. Oxidate and Agclor 310 were ineffective in controlling either *P. infestans* or *P. erythroseptica*. All tested phosphorous acid materials provided complete control of both tested pathogens in 2005 and in 2006. In a second test, less than labeled rates provided less than complete control of *P. infestans*. Phosphorus acid materials have potential for post-harvest control of late blight and pink rot. (*Plant Pathology Journal* 7 (1): 50-53, 2008; doi: 10.3923/ppj.2008.50.53)

### **Prospects for Biocontrol of Brown Rot Disease of Potato *in vitro* and under Greenhouse Conditions**

Amira A. El-Fallal and Zeiad Moussa

This investigation is a trial to biocontrol of brown rot disease of potato by basidiomycetes, wheat straw and spent mushroom straw. Bird's nest *Cyathus stercoreus* is firstly recorded in Egypt. It was found growing on manured soil at New Damietta. It is identified and isolated from its fruit body. Eight basidiomycetes including *C. stercoreus* were tested to antagonize *Ralstonia solanacearum* (causal agent of brown rot disease of potato) *in vitro*. All of these fungi inhibited the growth of *R. solanacearum* and the largest inhibition zones were recorded

with *Cyathus stercoreus* Egyptian strain and *Agaricus campester* Egyptian strain. Extract from the *Cyathus stercoreus* mycelia was studied using Thin Layer Chromatography (TLC), Infrared (IR) spectroscopic analysis and Nuclear Magnetic Resonance (NMR). The study suggested that the polysaccharide of this fungus has the antibacterial activity. *C. stercoreus* was selected to green house study. Water extract filtrate of raw wheat straw (wers) and of spent mushroom straw (wess) of three *Pleurotus* sp. were tested to inhibit *R. solanacearum* growth by applying disc-filter paper method. RSE filtrate and all the other three filtrates inhibited *R. solanacearum* growth. Water extract of *Pleurotus columbinus* spent wheat straw (wess) had the largest inhibition zone, so, it was selected to further study. In greenhouse experiment, the previous selected factors were used to estimate their ability to biocontrol *R. solanacearum*. The reported results revealed that the mycelial suspension of *C. stercoreus* (Css) had the best effect in reduction of the disease, plant health and plant production. Consequently, this is the first report of using *C. stercoreus* in biological control of plant diseases. Using of spent straw and raw straw powders and their water extracts had good results in controlling the disease and plant productivity. (*Plant Pathology Journal* 7 (1): 54-64, 2008; doi: 10.3923/ppj.2008.54.64)

## **Control of Clavicipitaceous Anamorphic Endophytes with Fungicides, Aerated Steam and Supercritical Fluid CO<sub>2</sub>-Seed Extraction**

A. Dan Wilson, Donald G. Lester and Brian K. Luckenbill

The effects of soil drenches with systemic fungicides on viability of clavicipitaceous anamorphic endophytes, non-choke inducing endosymbiotic fungi of the genus *Neotyphodium* that systemically infect grasses, were tested in endophyte-infected seedlings of *Hordeum brevisubulatum* subsp. *violaceum*, *Lolium perenne* and *Festuca arundinacea* germplasm. None of the endophytes of these grasses were sensitive to benomyl at 3-5 ppt. The endophyte of *H. brevisubulatum* was sensitive only to propiconazole. The viability of the *F. arundinacea* endophyte (*N. coenophialum*) was reduced, but not completely eliminated by prochloraz, imazalil and propiconazole in the 2-3 ppt range. Aerated-steam seed treatments at 60 C for 5 min were effective in reducing viability of the perennial ryegrass endophyte (*N. lolii*) by 83%, although some reduction in seed germination and negative growth effects were observed. A novel disease-control method, supercritical fluid carbon dioxide extraction (SFE-CO<sub>2</sub>), for the elimination of *Neotyphodium*-endophytes in grass seeds is reported here for the first time. The endophyte *N. lolii* was completely controlled in seeds of *L. perenne* cv. Ellett by

extracting the seeds at 400 atmospheres for 10 or 20 min. Only minor reductions in seed germination resulted from the SFE-CO<sub>2</sub> seed-extraction treatment and no appreciable effects on growth of seedlings or mature plants were observed. Several potential applications of these disease-control technologies for seed-borne fungal pathogens are discussed. (*Plant Pathology Journal* 7 (1): 65-74, 2008; doi: 10.3923/ppj.2008.65.74)

### **Propagation of the CABMV from Infected Seeds in Three Zones of Burkina**

B.J. Néya, J. Zabré, R.J. Millogo, S. Guinko and G. Konaté

The effect of contamination of cowpea seeds by *Cowpea aphid borne mosaic virus* (CABMV) on disease development was studied in three agro-ecological zones of Burkina Faso. Seed contamination is the only source of primary infection by CABMV in the field while aphid vectors are responsible for secondary infections. Two cowpea varieties with different seed contamination rate (0, 0.05, 0.25, 0.5, 1 and 5%) were used. The infected plants were recorded every week from the tenth Day After Sowing (DAS) and over seven weeks. In the same way, populations of aphids were evaluated in plots at 30 DAS. There was a difference between the average numbers of insects from one location to another. The initial contamination rate of cowpea seeds by the CABMV played a leading important role in epidemics development according to the areas. With the variety Local Gorom, the secondary infections were early and fast in Niangoloko zone. In Sapouy area, the secondary infections were lower and belated. In the centre zone the impacts were lower than those of Sapouy. In the case of variety *Kvx61-1*, the secondary infections were medium for all initial contamination rates even in years of great epidemics. For each year, any difference of impact rate had not got between the different seeds upset contamination levels *Kvx61-1* behaved well in the three localities even the west where aphids appear precociously and densely. (*Plant Pathology Journal* 7 (1): 75-84, 2008; doi: 10.3923/ppj.2008.75.84)

### **Investigation of Biological Properties, Double-Stranded RNA Patterns and Antigen Concentration in Satsuma Owari Mandarins Infected with *Citrus tristeza virus* in Aegean Region of Turkey**

Savas Korkmaz, Serkan Onder and Mustafa Gümüs

In this study, biological properties and dsRNA patterns of nine Aegean region Turkish isolates of *Citrus tristeza virus* were investigated. In addition, CTV



antigen concentration and effect of tissue sampling time taken from naturally affected Satsuma Owari mandarin plant grown in the field of Balikesir province were also determined. The results indicated that nine isolates graft inoculated to a set of indicator plants did not show any symptoms on sour orange, grapefruit and sweet orange plants or did not cause any stunting when compared with healthy control. However, all isolates showed vein clearing symptoms on Mexican lime. All nine strains showed full length of major dsRNA band of  $13.3 \times 10^6$  Da MW on Madam Vinous plant. Moreover, they showed three full lengths of major or minor dsRNA bands with 2.0, 0.8 and  $0.5 \times 10^6$  MW. All four different citrus varieties inoculated with KE-5 strain showed full length of major dsRNA. The additional two dsRNA of 0.8 and  $0.5 \times 10^6$  MW were also detected as clear as full-length of dsRNA in four hosts, but recovery of these bands were less visible in sour orange. The highest ELISA value was obtained in March and April and then steadily decreased until September and then again gradually increased through December. ELISA value was the lowest in September. DsRNA recovery from a single isolate of CTV EK-2 grown in the field was good in March, April, May and June and it was poor in January, February, July, August, November and December. No dsRNA band was detected in September and October. (*Plant Pathology Journal* 7 (1): 85-91, 2008; doi: 10.3923/ppj.2008.85.91)

### **Detection of Phytoplasma Associated with Periwinkle Virescence in Egypt**

Ayman F. Omar, Amero A. Emeran and Jehan M. Abass

Little leaves, shortened internodes, virescence and witches' broom symptoms were observed affecting periwinkle plants growing in Kafr-el-sheikh governorate. These plants were examined for phytoplasma infections by electron microscopy. Phytoplasma appearing as spherical to ovoid structures of variable sizes were observed in phloem sieve tubes in diseased plants samples but not in symptomless plants samples by TEM. PCR-amplified phytoplasma of 16S rDNA using primers P1/P7 and PF2/PR2 was employed for the detection and identification of the phytoplasma associated with periwinkle plant. The Egyptian Phytoplasma Virescence (EPV) detected in diseased periwinkle (GenBank accession No. EF546439) was identified as members of aster yellow phytoplasma group (16SrI group) (*Candidatus* phytoplasma asteris). This is the first report on the occurrence of phytoplasma diseases in Egypt. (*Plant Pathology Journal* 7 (1): 92-97, 2008; doi: 10.3923/ppj.2008.92.97)

## **Seed-Borne Infection of Farmer-Saved Maize Seeds by Pathogenic Fungi and Their Transmission to Seedlings**

I. Somda, J. Sanou and P. Sanon

Seed-borne infection of maize (*Zea mays* L.) by fungal pathogens was studied using 22 seed samples of maize collected from different locations in Burkina Faso. The moist blotter test was used to detect fungi on seeds. Experiment was also performed on seedlings raised from naturally infected maize seed samples in order to evaluate the transmission of fungi from seeds to seedlings. Ten pathogenic fungi were recorded on seeds and consisted of *Acremonium strictum* (infection ranging from 2 to 96%), *Bipolaris maydis* (1 to 30%), *Botryodiplodia theobromae* (1 to 17%), *Colletotrichum graminicola* (2 to 8%), *Curvularia* sp. (1 to 39%), *Exserohilum rostratum* (1 to 13%), *Fusarium moniliforme* (38 to 99%), *F. equiseti* (1 to 15%), *F. pallidoroseum* (1 to 23%) and *Phoma* sp. (2 to 50%). *Aspergillus flavus* (1 to 99%), *A. niger* (1 to 99%), *Cladosporium* sp. (1 to 93%), *Penicillium* sp. (12 to 100%) and *Rhizopus* sp. (1 to 51%) were the saprophytic fungi detected. Rates of seedling infection by fungal pathogens were low. Seed samples with 30% infection by *B. maydis*, 8% infection by *C. graminicola* and 26% infection by *Phoma* sp. resulted in 2, 2 and 16% leaf infections, respectively. (*Plant Pathology Journal* 7 (1): 98-103, 2008; doi: 10.3923/ppj.2008.98.103)

## **Effect of Surfactants on Bioherbicidal Activity of *Alternaria helianthi* on Multiple-Seeded Cocklebur**

D. Sanyal, P.C. Bhowmik and H.K. Abbas

Multiple-seeded cocklebur (*Xanthium strumarium* L.) is a biotype which has different morphology and higher seedling production ability than common cocklebur. Greenhouse studies were conducted to investigate the bioherbicidal activity of *Alternaria helianthi* (Hansf.) Tubaki and Nishih. on multiple-seeded cocklebur as affected by various rates of Tenkoz COC® (crop oil concentrate), Activator 90® (non-ionic surfactant), BAS 9050 0 S® (methylated oil), Silwet L-77® (organosilicone surfactant) and Top film® (natural based surfactant). Taking X as the recommended rate for each surfactant, 0-X, ¼-X, ½-X, X and 2-X rates were used for each of the surfactants. Surfactants were added to the conidial suspension of *A. helianthi*. Each surfactant rate was also applied with sterile water

without any *A. helianthi* spore. Treated plants were kept in the dew chamber for 6 h before transferring to the greenhouse. At the end of the experiment (two weeks after treatment), plant shoots were clipped at the soil surface and the fresh weights were determined. *Alternaria helianthi* resulted in significant reduction in fresh weight of multiple-seeded cocklebur as compared to the plants treated without the fungus. Among five surfactants, Activator 90® and Silwet L-77® had significant effects on the bioherbicidal activity of *A. helianthi* in reducing fresh weight of multiple-seeded cocklebur. Fresh weight decreased with increase in surfactant rates. Our data demonstrate that *A. helianthi* control multiple-seeded cocklebur more with higher rates of Activator 90® and Silwet L-77®. (*Plant Pathology Journal* 7 (1): 104-108, 2008; doi: 10.3923/ppj.2008.104.108)

## **Diversity of the Rice Blast Pathogen Populations in Ghana and Strategies for Resistance Management**

S.K. Nutsugah, J.K. Twumasi, J. Chipili, Y. Séré and S. Sreenivasaprasad

The present study describes the outputs of a collaborative research programme funded by the UK's Department for International Development-Crop Protection Program to investigate the genetic (lineages) and pathogenic (pathotypes) diversity of the blast fungus populations and characterize the key sites suitable for resistance screening. Seventy-one *Magnaporthe grisea* isolates were collected from seven regions where rice is grown, representing blast populations in Ghana. Following molecular characterization, these isolates were grouped into four distinct lineages designated as GH-1, GH-2, GH-3 and GH-4 and 25 pathotypes. GH-1 was the major lineage comprising 52% of all the isolates and was present across the country on up to 24 rice cultivars. GH-2 comprising of 30% of the isolates sampled was restricted in distribution mainly from Hohoe area on up to seven cultivars. GH-3 consisted of six isolates from Western, Eastern and Central Regions while GH-4 consisted of two isolates from Nyankpala in Northern Region. Occurrence of blast pathogen on wild rice and weed hosts has been observed and their potential impact needs to be considered in blast/weed management. Baseline data new to Ghana on the diversity and distribution pattern of the blast pathogen populations have been established and key sites identified. Adaptive research is continuing to develop technologies suitable for long-term pathogen monitoring, identify sources of resistance and develop appropriate blast management strategies. (*Plant Pathology Journal* 7 (1): 109-113, 2008; doi: 10.3923/ppj.2008.109.113)

## **Separate and Combined Effects of *Fusarium oxysporum* f. sp. *tracheiphilum* and *Meloidogyne incognita* on Growth and Yield of Cowpea (*Vigna unguiculata* L. Walp) Var. Moussa-Local**

A.O. Ogaraku

A study was undertaken to determine the separate and combined affects of *Fusarium oxysporum* f. sp. *tracheiphilum* and *Meloidogyne incognita* on growth and yield of cowpea variety Moussa-local. It was observed that growth and yield components of the cowpea were generally reduced in all treatments as compared to that of control. Single infection with only nematode caused more growth and yield reductions than infection with only fungus. Reduction in the growth and yield components were generally higher in simultaneous infection than infection with either of the pathogens. Growth and yield reduction in combined infection did not vary significantly from those caused by successive infection where either of the pathogens was made to precede the other. Infection with both pathogens caused significant increases in the root galls, number of eggs and juveniles when compared with infection with nematode only. (*Plant Pathology Journal* 7 (1): 114-117, 2008; doi: 10.3923/ppj.2008.114.117)

## **Association of Selective Deposition of (1→3)-β-Glucan in Floral Tissues with Restricted Movement of Turnip Vein-Clearing Virus in *Arabidopsis*: A Possible Mechanism for Non-Seed Transmission**

Robert T. Lartey, Kajal Ghoshroy and Soumitra Ghoshroy

In this research we used *Turnip vein clearing virus* (TVCV) to study viral non-transmission in *A. thaliana* as a step toward developing a management strategy against seed transmissible viruses. Confocal laser scanning, light, transmission and scanning electron microscopy and immunolabeling techniques were used to study the pattern of the viral movement in reproductive parts of the systemically infected plant. The virus was located in all parts of the flower except ovules and pollen grains. The viral movement was thus confined to the junction of ovule and ovule stalk in the siliques and outside the pollen grains in anther tissue. Subcellular localization of (1→3)-β-glucan (callose) indicated selective deposition of this substance in plasmodesmata at the junction of ovule and ovule stalk as well as exine layer of pollen grains. The callose deposition was not observed in uninoculated and mock inoculated control plants. We propose that the observed

(1→3)- $\beta$ -glucan may be involved in blockage of viral movement into ovules and pollen grains and result possibly in non-seed transmission of TVCV in *A. thaliana*. (*Plant Pathology Journal* 7 (2): 120-130, 2008; doi: 10.3923/ppj.2008.120.130)

### **Mycelial Compatibility Group and Pathogenicity Variation of *Sclerotinia sclerotiorum* Populations in Sunflower From China, Canada and England**

Ziqin Li, Min Zhang, Yingchun Wang, Ru Li and W.G. Dilantha Fernando

This study ascertained mycelial compatibility groups (MCGs) and pathogenicity variations of *Sclerotinia sclerotiorum* isolates sampled in sunflower from China, Canada and England. For this purpose, 205 isolates (5 from England, 33 from Canada and 167 from China) were grouped in 39 MCGs. Out of 39 MCGs, 64% were represented each by a single isolate. *Sclerotinia* isolates from the three countries' were incompatible between each other. Differences among MCGs were observed by comparing their differences in radial growth, sclerotial yield on PDA and detached leaves, aggressiveness and production of oxalic acid and total acids. Significant differences were found in radial growth, sclerotial yield, aggressiveness, production of oxalic acid and total acids within and among MCGs ( $p < 0.001$ ) regardless of their geographic origins. Correlation analysis indicated that *S. sclerotiorum* aggressiveness was positively related to oxalic acid production ( $r = 0.739$ ,  $p < 0.01$ ) and negatively related to pH ( $r = -0.436$ ,  $p < 0.01$ ), but not to the radial growth and the yields of sclerotia on PDA and detached sunflower leaves. Significantly, sclerotial yield on PDA was positively correlated to the sclerotia weight produced on detached sunflower leaf. There was a highly negative relationship between pH values (negatively indicated total acids secretion) and oxalic acid production ( $r = -0.669$ ,  $p < 0.01$ ), suggesting that oxalic acid contributes most to the total production of acids released by *S. sclerotiorum*. (*Plant Pathology Journal* 7 (2): 131-139, 2008; doi: 10.3923/ppj.2008.131.139)

### **Role of Plant Host in Determining Differential Responses to *Ralstonia solanacearum* and *Glomus mosseae***

M.M. Tahat, O. Radziah, S. Kamaruzaman, J. Kadir and N.H. Masdek

A pot study was aimed to investigate the role of tomato in determining differential response to bacterial wilt causal agent *Ralstonia solanacearum* pathogen and

arbuscular mycorrhizal fungi (AMF) *Glomus mosseae*. Disease severity was measured after 10, 20 and 30 days of plant growth. The pathogen and dual treatment (*R. solanacearum* with *G. mosseae*) were not significantly different at the end of this experiment. Soil pH was greatly influencing the pathogen and AMF microbe. *Glomus mosseae* mycorrhizosphere was more alkaline (pH 5.9) compared to the pathogen mycorrhizosphere (pH 4.9). The concentration of bacterial cell in the *R. solanacearum* soil was not different from the dual treatment after 60 days of plant growth. Spore germination was influenced by the interaction between the soil pathogen and AMF. Spores number in the dual treatment at 60 days was less than the original number added. Root colonization percentage in *G. mosseae* (61%) was significantly more than the dual treatment (16%). This provide an evidence about the role of plant host in increasing the spores germination influenced by many substances produced by the host root (root exudates). The results demonstrated that the role of plant in determination the relationship between soil-borne pathogen and antagonistic microbe was critical. (*Plant Pathology Journal* 7 (2): 140-147, 2008; doi: 10.3923/ppj.2008.140.147)

### **Suppressive Effect of Mature Compost of Date Palm By-Products on *Fusarium oxysporum* f. sp. *albedinis***

K. Chakroune, M. Bouakka, R. Lahlali and A. Hakkou

The aim of this study was to evaluate the possibility of exploiting mature compost of date palm by products in order to control *Fusarium oxysporium* f. sp. *albedinis* disease (Foa) commonly known as Bayoud. The obtained results showed that applying compost in mixture with peat and vermiculite S2 reduced significantly the losses of date palm seedlings of susceptible cultivar Bouffaggousse Gharasse due to the presence of Foa. However, the use of mixture substrate containing only peat and vermiculite S1 resulted in the death of 90% of the palm seedlings, as compared to non-infested S1. Also, the Foa population density and seedlings date palm mortality dropped in substrate mixtures S4 and S5 as the mature compost increased in soil substrate independently of date palm cultivar. The suppressive effect of compost seems reinforced by its richness of microorganisms having an antagonistic activity against Foa such as *Aspergillus*, *Penicillium* and *Bacillus*. (*Plant Pathology Journal* 7 (2): 148-154, 2008; doi: 10.3923/ppj.2008.148.154)

## **Characterization of *Pepper yellow vein mali virus* in *Capsicum* sp. in Burkina Faso**

Fidèle Tiendrébéogo, V.S. Edgar Traoré, Nicolas Barro, Alfred S. Traoré, Gnissa Konaté and Oumar Traoré

The aetiology of a disease characterized by severe leaf curling and leaf distortion symptoms in peppers was studied in Burkina Faso. Surveys were conducted in fields planted with sweet pepper (*Capsicum annuum*) and hot pepper (*Capsicum frutescens*) to collect leaf samples and assess disease incidence. Collected samples were analyzed using biological tests (mechanical inoculation and whitefly transmission), triple-antibody-sandwich-enzyme linked-immunosorbent assay (TAS-ELISA), Polymerase Chain Reaction (PCR) and sequence analysis. Mechanical inoculation tests were negative while transmission by the whitefly *Bemisia tabaci* led to infection rates of more than 80%. TAS-ELISA and PCR tests indicated that symptoms observed were associated with begomovirus infections. Involved virus isolates showed pairwise nucleotide identity of more than 99% with *Pepper yellow vein mali virus* (PepYVMV) and formed with this virus a distinct clade in phylogenetic analyses. Altogether, these results indicated that PepYVMV was the causal agent of leaf curling symptoms in peppers. (*Plant Pathology Journal* 7 (2): 155-161, 2008; doi: 10.3923/ppj.2008.155.161)

## **Genomic Diversity of *Ralstonia solanacearum* Strains Isolated from Banana Farms in West Malaysia**

R. Khakvar, K. Sijam, M.Y. Wong, S. Radu, J. Jones and K.L. Thong

Pulse-Field Gel Electrophoresis (PFGE) and BOX-PCR patterns were used to determine the phylogenetic relationships among 32 strains of *Ralstonia solanacearum* collected from banana farms in West Malaysia. Eighty two percent (25 stains) were biovar 3 and the remainders were biovar 4. Cluster analysis based on PFGE and BOX-PCR fingerprinting showed that there was significant genetic variation among all strains within this species in West Malaysia. Patterns of DNA fingerprinting in both methods were significantly correlated with sampling sites and revealed the regional similarity among the strains. The PFGE patterns varied from 78 to 99% while BOX-PCR patterns showed a high degree of variation among *R. solanacearum* strains. Genomic fingerprinting by PFGE also revealed one that was significantly correlated to biovar type while BOX-PCR pattern analysis was unable to differentiate various biovars. This study clearly

showed that *R. solanacearum* strains were phylogenetically similar within a region but diverse between regions despite biovar designation. (*Plant Pathology Journal* 7 (2): 162-167, 2008; doi: 10.3923/ppj.2008.162.167)

### **Age-Related Resistance in Commercial Varieties of *Solanum tuberosum* to the Late Blight Pathogen, *Phytophthora infestans***

S.D. Mutty and Nawsheen T. Hossenkhan

This study reports the occurrence of Age-Related Resistance (ARR) in the potato-*P. infestans* pathosystem. Six potato varieties with different degrees of resistance to *P. infestans* were evaluated on the basis of disease severity according to the widely-used Malcolmson's scoring scale at 4, 8 and 12 weeks after plant emergence. The results show that, out of the six varieties, those exhibiting high to moderate levels of resistance to late blight became consistently more resistant as they grew older, while the effect was less pronounced on the two remaining susceptible varieties. Those six varieties exhibited their maximum level of resistance at 12 weeks after plant emergence, when they were just at the bud stage, making the transition to flowering. It is possible that blight resistance in potato plants might be a developmentally regulated response and as the potato plant makes the transition from vegetative to reproductive growth, the re-wiring of certain developmental pathways induces the expression, or results in the accumulation of, certain genes which might also play an important part in defense responses. The data presented in this study emphasizes the importance of considering the age of potato plants when challenged in screening for resistance as well as in determining the most effective timing for use of chemical controls. (*Plant Pathology Journal* 7 (2): 168-173, 2008; doi: 10.3923/ppj.2008.168.173)

### **Efficacy of Essential Oils on the Conidial Germination, Growth of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc and Control of Postharvest Diseases in Papaya (*Carica papaya* L.)**

Laura Leticia Barrera-Necha, Silvia Bautista-Baños, Hilda Elizabeth Flores-Moctezuma and Abel Rojas Estudillo

The efficacy of nine essential oils were investigated *in vitro* on conidial germination and mycelial growth inhibition of *Colletotrichum gloeosporioides* isolate from papaya (*Carica papaya* L.). In general, better antifungal effect was observed with



*Cinnamomum zeylanicum* and *Syzygium aromaticum* oils which had strong inhibition of conidial germination of *C. gloeosporioides* at 50, 100, 150, 200 and 250  $\mu\text{g mL}^{-1}$  and a dose dependent inhibition mycelial growth was caused by these oils. *Teloxys ambrosioides*, *Mentha piperita* and *Ruta chalepensis* oils exhibited a moderate action at 150, 200 and 250  $\mu\text{g mL}^{-1}$  on conidial germination and mycelial growth inhibition. *Allium sativum*, *Citrus aurantifolia* and *Eucalyptus globulus* oils had no antifungal activity at different concentration. Taking into account the *in vitro* results, *C. zeylanicum* and *S. aromaticum* oils were evaluated on papaya fruit during storage at ambient temperature and 14°C. The lowest infection percentage were for papaya fruits treated with *S. aromaticum* at 50  $\mu\text{g mL}^{-1}$  at both temperature tested, nevertheless did not overcome the activity of synthetic fungicide. After storage at both temperature, values of Soluble Solids Content (SSC) was not significantly different. *S. aromaticum* oils may be a possibility to control *C. gloeosporioides* of papaya fruit. (*Plant Pathology Journal* 7 (2): 174-178, 2008; doi: 10.3923/ppj.2008.174.178)

### **Effect of Humic Acid Applications on the Root-Rot Diseases Caused by *Fusarium* spp. on Tomato Plants**

Fahri Yigit and Murat Dikilitaş

A study was conducted to elucidate the relations between the effects of humic acid applications and incidents of root rot diseases of tomato in greenhouse soils contaminated with soil-borne pathogens. For this purpose, commercially available powder preparations containing 80% humic acid and fulvic acid were used in the experiment. After the application of three different doses (80, 160, 240 mg active ingredient, a.i.,  $\text{plant}^{-1}$ ), with five replications, healthy and diseased plants were counted and root weights were recorded to determine the disease development followed by fruit maturity. The disease percentage of the control group was found as 30.4 while those of the treated groups were 29.6, 30.8 and 34.4 with respect to doses of 80, 160 and 240 mg a.i.  $\text{plant}^{-1}$ , respectively. Average root fresh weight in the control group was found 110 g  $\text{plant}^{-1}$  while those of the treated groups were found as 135, 156, 215 g  $\text{plant}^{-1}$ , respectively. The results clearly showed that the application of humic acid caused an increment in root fresh weight while making plants to susceptible to the root rot pathogens. (*Plant Pathology Journal* 7 (2): 179-182, 2008; doi: 10.3923/ppj.2008.179.182)

## **Mycotoxigenic Potential of Ten *Fusarium* species Grown on Sorghum and *in vitro***

Thomas Isakeit, Louis K. Prom, Michael Wheeler, Lorraine S. Puckhaber and Jinggao Liu

The objective of this study was to determine the mycotoxigenic potential of 12 *Fusarium* isolates (10 species), including six isolates (4 species) from sorghum. The species were: *F. thapsinum*, *F. semitectum*, *F. proliferatum* and *F. chlamydosporum* isolated from molded sorghum seed; *F. poae*, *F. graminearum* and *F. sporotrichioides* from barley seed with *Fusarium* head blight; *F. acuminatum* from wheat seed; *F. verticillioides* from infected corn seed; and *F. nygamai* isolated from soil. Fumonisin and zearalenone concentrations were measured following incubation on autoclaved sorghum seed for 21 days at 25°C, while fusaric acid was measured in mycelia harvested from Czapek Dox broth cultures. *F. thapsinum* (SC8 and CS121) and *F. semitectum* (SC7) produced fusaric acid only (4.59-64.13 mg g<sup>-1</sup>). *F. graminearum* (KB172) and *F. semitectum* (CS152) produced zearalenone only (73.4 and 799.3 µg g<sup>-1</sup>, respectively). *F. proliferatum* (CS183), *F. verticillioides* (TX02) and *F. nygamai* produced both fumonisin (1.92-6.05 µg g<sup>-1</sup>) and fusaric acid (39.4-234.17 mg g<sup>-1</sup>). *F. poae* (KB652), *F. acuminatum* (Ark), *F. chlamydosporum* (CS102) and *F. sporotrichioides* (KB662) did not produce any of these three mycotoxins. Five of the six *Fusarium* isolates (three species) isolated from sorghum had mycotoxigenic potential. *Fusarium* spp. naturally occurring on sorghum in the field have the potential to contribute to mycotoxin contamination, either singly or in combination. (*Plant Pathology Journal* 7 (2): 183-186, 2008; doi: 10.3923/ppj.2008.183.186)

## **Variation of Lipopolysaccharide among the Three Major *Agrobacterium* Species and the Effect of Environmental Stress on the Lipopolysaccharide Profile**

H.H. Arafat, K. Tanaka, H. Sawada and K. Suzuki

Lipopolysaccharide (LPS) is a variable component among the bacterial species as well as strains of a single species and this characteristic is helpful for discrimination between strains. However, we have only limited information about LPS variation and influence by environment in *Agrobacterium* strains. In this study, we analyzed variation of lipopolysaccharide (LPS) among 34 *Agrobacterium* strains; 9 strains of *A. tumefaciens*, 15 strains of *A. rhizogenes*, 9 strains of *A. vitis* and one

*A. rubi* strain. Most of the *A. tumefaciens* strains and every *A. rhizogenes* strains had high and low molecular weight LPS molecules (LPS I and LPS II, respectively). On the contrary, every *A. vitis* strains and two exceptional *A. tumefaciens* strains lacked LPS I but had a single LPS II band. The LPS profiles were stable phenotype in the *Agrobacterium* strains. Abiotic stresses such as high salinity, high and low pH and high and low temperature were given to representative strains in each species. Only a little alternation in the LPS profiles was observed under the stress conditions except the high temperature to LPS I. Cultivation at 35°C or higher resulted in a significant size reduction of LPS I in *A. tumefaciens* C58 strain down to the size similar to that of LPS II which attenuated the tumor formation. On the contrary, cultivation at the high temperature induced the exceptional *A. tumefaciens* strain MAFF 03-01001 to synthesize LPS I, which was absent at lower temperature in the strain. This phenomenon has never been observed so far at least in the family *Rhizobiaceae*. (*Plant Pathology Journal* 8 (1): 1-8, 2009; doi: 10.3923/ppj.2009.1.8)

### **Effect of Combined Use of *Bacillus subtilis* CA32 and *Trichoderma harzianum* RU01 on Biological Control of *Rhizoctonia solani* on *Solanum melongena* and *Capsicum annuum***

S. Abeysinghe

A combination of two compatible biological control agents, *Bacillus subtilis* CA32 and *Trichoderma harzianum* RU01, both antagonistic to the pathogen *Rhizoctonia solani*, was used to control damping-off in *Solanum melongena* and *Capsicum annuum*. Radial growth of the mycelium of *R. solani* was inhibited by *T. harzianum* RU01 in dual Petri plate assay. *T. harzianum* RU01 was capable to invading the whole surface of the pathogen colony, sporulating on it and suppress the production of sclerotia of *R. solani*. Microscopic studies showed the hyphae of *R. solani* surrounded by the *T. harzianum* RU01 and subsequent disintegration. *B. subtilis* CA32 produced a zone of inhibition only with the pathogen and no signs of antagonism between the bacteria and *T. harzianum* RU01 on dual Petri plate assay. Significant plant protection was achieved when either *B. subtilis* added to the seeds or *T. harzianum* added to soil. However, when combine application of biocontrol agents, seed bacterization and *T. harzianum* application to soil, significantly enhanced the plant protection from *R. solani*. Soil application of *B. subtilis* and seed application of *T. harzianum* either singly or in combination did not protect from *R. solani* infection indicating that the importance of mode of application of biocontrol agents. (*Plant Pathology Journal* 8 (1): 9-16, 2009; doi: 10.3923/ppj.2009.9.16)

## ***In vitro* Antifungal Activity of Essential Oils and Their Compounds on Mycelial Growth of *Fusarium oxysporum* f. sp. *gladioli* (Massey) Snyder and Hansen**

L.L. Barrera-Necha, C. Garduño-Pizaña and L.J. García-Barrera

The increasing recognition and importance of phytopathogenic fungi, the difficulties encountered in their control and the increase in resistance to antifungal have stimulated the search for natural alternatives. The antifungal effects of essential oils and their compounds were investigated on mycelial growth inhibition bioassays of *Fusarium oxysporum* f. sp. *gladioli*. The essential oils have been used empirically. In general, a significant antifungal effect was observed with *Cinnamomum zeylanicum*, *Thymus vulgaris* and *Syzygium aromaticum* oils which had total inhibition at 100, 150, 200, 250 and 300 ppm. *Teloxys ambrosioides*, *Mentha piperita* and *Citrus aurantifolia* oils exhibited a dose dependent inhibition on mycelial growth to increase the dose of 100 at 300 ppm. While *Allium sativum*, *Capsicum* sp., *Ruta chalepensis* and *Eucalyptus globulus* oils had no antifungal activity at different concentration tested. All compounds with the exception of cineole had a fungicide or fungistatic effect. (*Plant Pathology Journal* 8 (1): 17-21, 2009; doi: 10.3923/ppj.2009.17.21)

## **Alkaline Seed-Bed: An Innovative Technique for Manifesting *Verticillium dahliae* on Fennel Seeds**

Khalid M. Ghoneem, Wesam I.A. Saber and Mohamed A. Elwakil

*Verticillium dahliae* attacks a wide range of plants including fennel causing a wilt disease. The fungus grows slowly on seeds when tested at the seed health laboratories. This habit character allows saprophytes to impair the fungal growth and interfere the identification on both Moist Blotters (MB) and the Deep-Freezing Blotters (DFB). Since, these two techniques are not efficient enough to detect this fungus, the researchers planned to search for an alternative technique for detecting this fungus. Soaking three layers of blotters used as seed-beds in water solutions alkalined with KOH or NaOH at pH 10 presents the optimum seed-bed condition for manifesting the fungus on seed. This seed-bed condition also suppress the growth of saprophytes, so as the fungus was transparently shown on seeds. The *in vitro* study presents pH 9.5 as the optimum condition for the growth, sporulation and maximum glucose coefficient of the fungus. So far, it is recommended to use the alkalined seed-bed when searching for *V. dahliae* on

fennel seed. (*Plant Pathology Journal* 8 (1): 22-26, 2009; doi: 10.3923/ppj.2009.22.26)

## **Epidemiology of Potato Blackleg in Warm Climate**

Nabil S. Farag, Ahmad A. Gomah and Naglaa A.M. Balabel

Potato (*Solanum tuberosum* L.) planted in warm climate in September, in Egypt, showed no above ground symptoms of blackleg. The harvested crop showed severe tuber collapse similar to that being produced by the soft rot disease. Tentative identification of the isolated bacteria revealed no affiliation to the soft rot bacterium *Erwinia carotovora* ssp. *carotovora*. Verification of identity was made by PCR that showed its close similarity, to *E. carotovora* ssp. *atroseptica* at 119 bp, compared to an authentic Dutch isolate PD 4202. The present study suggested that the quality of water phase in the field soil may play an important role in rot progress at the time of harvest. Tonicity, cation(s) content and pH of the water phase of soil, suspending the bacterial inoculum, were found important in this regard. The rot increased under hypertonic stress ( $10\text{ g}^{-1}$  NaCl) was attributed to a greater proliferation of bacteria on the expense of nutrients withdrawn from tuber cells. Further increase ( $20\text{ g}^{-1}$  NaCl) in tonicity, however, decreased tuber rot, indicating negative correlation between the aggressiveness of strain to potato tubers and their osmotic tolerance. Pathogen suspended in hypotonic solution produced greater rot possibly due to greater invasion by bacteria and/or maximization of the intercellular spaces following cell turgidity. The monovalent and divalent cations at approximately similar osmotic strength decreased the rot symptoms. This effect may be attributed to their effect on a group of depolymerases with different optimal conditions and/or their effect on changing optimal pH for pectinolysis. The tuber rot was recorded over a wide range of pH. Further investigations are needed to study in depth other edaphic factors related to epidemiology of blackleg disease in warm climate. (*Plant Pathology Journal* 8 (1): 27-31, 2009; doi: 10.3923/ppj.2009.27.31)

## **Identification of *Puccinia pimpinellae* on Anise Plant in Egypt and Its Control**

Wesam I.A. Saber, Khalid M. Ghoneem, Mohammed M. El-Metwally and Mohamed A. Elwakil

An emerging problem for the wider adoption of anise plantation in Egypt is the damage caused by the rust fungus. The detailed description and taxonomic studies

(using light and scanning electron microscopy) show that such an obligate parasite fungus (*Puccinia pimpinellae*) is autoecious microcyclic (uredinial-telial stage only). Among tested Apiaceae plants, the host range test proved the specificity of the rust fungus to anise. To the researcher's knowledge, this is the first investigated record of a rust fungus on *Pimpinella anisum* plants in Egypt. The effectiveness of some plant resistance elicitors and two active chitinase producers; *Bacillus subtilis* Bio4 and isolated *Trichoderma harizianum* CH<sub>4</sub> (both of them recorded the highest clear zone/colony size ratio on chitin agar plates) in controlling anise rust disease and on growth and yield of anise were evaluated in two successive growing seasons. Spraying chitosan at 1000 ppm was the most potent in reducing Disease Severity (DS) and Incidence (DI) as well as improving plant height, chlorophyll content, inflorescence No. plant<sup>-1</sup> (74.2 and 76), 1000-fruit weight (2.94 and 2.83 g) and anise yield (646.8 and 670.0 kg fed<sup>-1</sup>), during both seasons. *B. subtilis* Bio4 and *T. harizianum* CH<sub>4</sub> showed moderate effect on the tested parameters. (*Plant Pathology Journal* 8 (2): 32-41, 2009; doi: 10.3923/ppj.2009.32.41)

### **Screening of Transgenic Tobacco for Resistance Against Cucumber mosaic virus**

K. Nadarajah, N.M. Hanafi and S.L. Tan

Coat protein (*CP*), Movement Protein (*MP*) and Overlapping (*OVG*) genes were isolated from a Malaysian *Cucumber mosaic virus* (CMV) isolate via RT-PCR and transformed into *Nicotiana tabacum* through *Agrobacterium tumefaciens*-mediated transformation. Out of the thirty six independently transformed lines developed from the three different genes and the mutants of *MP* and *OVG*, five lines were tested for resistance against CMV by challenge inoculations using three different concentrations (1:10, 3:10 and 5:10) of CMV macerated in 0.1 M sodium phosphate buffer (pH 7.0). The transgenic lines exhibiting complete resistance remained symptomless even when re-inoculated with 1:10 concentration of virus. The level of viral RNA accumulation in inoculated leaves was significantly (at least 2-3 times) lower compared to the control untransformed plants. The upper leaves which were analysed for systemic spread of the infection had much lower levels of viral RNA accumulation compared to the inoculated leaves. Amongst the three genes and two mutant lines that were generated in this study, we found that the *CP* and *MP* genes were able to provide a better level of resistance to the plants compared to the overlapping gene. (*Plant Pathology Journal* 8 (2): 42-52, 2009; doi: 10.3923/ppj.2009.42.52)

## **Plant Resistance to TSWV and Seed Accumulation of Resveratrol within Peanut Germplasm and its Wild Relatives in the US Collection**

M.L. Wang, D.L. Pinnow, N.A. Barkley and R.N. Pittman

Biotic and abiotic stress may induce peanut plants to produce a high amount of resveratrol. The relationship between an individual plant's response to biotic stress caused by *Tomato spotted wilt virus* (TSWV) and the accumulation of resveratrol in the seed was investigated. Twenty peanut accessions and six wild relatives were selected from the US peanut germplasm collection and planted with two replicates. Individual plant response to natural-TSWV infection was observed and recorded in the field. Leaf tissues from each individual plant were collected and tested by an Enzyme-Linked Immunosorbent Assay (ELISA) using specific antiserum for TSWV. Seeds harvested from individual plants were used for quantification of resveratrol by High Performance Liquid Chromatography (HPLC) analysis. Extensive resveratrol variation in the seeds was detected among TSWV negative and positive plants. Among the accessions evaluated in this study, the specific genotype of each individual definitely played a major role on the capability for synthesis and accumulation of resveratrol. However, the synthesis and accumulation of resveratrol within an accession may not only be affected by a plant's response to TSWV, but also by other biotic and abiotic stress that an individual plant encounters in its environment. (*Plant Pathology Journal* 8 (2): 53-61, 2009; **doi:** 10.3923/ppj.2009.53.61)

## **Characterization of a Novel Far-Eastern Potato Virus Y Isolates**

Yury G. Volkov, Nadezhda N. Kakareka, Larissa A. Balabanova, Zinaida N. Kozlovskaya and Mikhail V. Sapotsky

Potato Virus Y (PVY) isolates differed in pathogenicity and molecular properties were found in potato and wild plants in the Far East of Russia. The results of linking research of nucleotide sequence similarity and polymorphism of P1 gene region and serological and biological assays suggested that the viruses originated from a recombination and/or host adaptation events involving the ordinary type virus (PVY<sup>0</sup>) that led to development of necrotic type virus (PVY<sup>N/NTN</sup>) properties in the Far-Eastern PVY isolates. (*Plant Pathology Journal* 8 (2): 62-67, 2009; **doi:** 10.3923/ppj.2009.62.67)

## **Physiological Race of *Fusarium oxysporum* F. sp. *Lycopersici* in Kurdistan Province of Iran and Reaction of Some Tomato Cultivars to Race 1 of Pathogen**

J. Amini

In this research, eleven isolates of *Fusarium oxysporum* were collected from tomato plants displaying wilt symptoms in fields in Kurdistan province. Race 1 of pathogen was obtained from Moscow Timiryazev Agricultural Academy in Russia. Pathogenicity of the collected isolates and race 1 of the pathogen were evaluated in glasshouse conditions. Pathogenicity tests and race determination were conducted using root-dip inoculation with different tomato cultivars, Bely naliv-241 (not resistant), Blagovest (resistant to race 1) and Benito (resistant to both races of 1 and 2). The experimental design was a completely randomized type with six replications (pots) containing two seedlings per pot. Disease severity was measured five weeks following inoculation by using a scale of 0 to 4. The criteria used to assess the response of different cultivars were; leaf disease index, plant height and vascular discoloration index. Results showed that Bely naliv-241 lacking any resistance gene wilted four weeks after inoculation, but cultivars Blagovest and Benito did not develop symptoms to any of the isolates tested. The reaction of race 1 and the Iranian isolates were similar in pathogenicity suggesting that all of the isolates belong to *Fusarium* f. sp. *lycopersici* race 1. Also, Reaction 23 tomato cultivars against to *F. o. f. sp. lycopersici* indicated that 6 of the them were resistant, 5 were intermediately resistant, 6 were tolerant, 3 were susceptible and the rest 3 were found to be very susceptible. (*Plant Pathology Journal* 8 (2): 68-73, 2009; doi: 10.3923/ppj.2009.68.73)

## **Using Arbuscular Mycorrhizal Fungi and *Rhizobium leguminosarum* Biovar *phaseoli* Against *Sclerotinia sclerotiorum* (Lib.) de Bary in the Common Bean (*Phaseolus vulgaris* L.)**

E. Aysan and S. Demir

In this study, the effects of Arbuscular Mycorrhizal Fungi (AMF) *Glomus mosseae* (Gm), *Glomus fasciculatum* (Gf) and *Rhizobium leguminosarum* biovar *phaseoli* (Rlp), which are the important members of rhizosphere and biological control agents, were examined on the patho-system of *Sclerotinia sclerotiorum* (Lib.) de Bary (Ss) and common bean. The colonization and nodulation of two biological control agents exhibited differences as a result of



reciprocal interactions of these items as well as the effect of the Ss. Nodulation of Rlp particularly decreased in triple inoculation. In addition, colonization of AMF significantly decreased in treatment of Ss+AMF than control AMF. Treatments of single inoculations of AMF and Rlp isolates reduced disease severity by 10.3-24.1%. It was determined that single biological control agents inoculations were more effective than dual inoculations (AMF+Rlp). When the morphological parameters of common bean were considered, all of the morphological parameters values were decreased in treatments which present pathogen isolate. Besides this, all biological control agents increased total contents of P and N in treated plants compared to the controls. (*Plant Pathology Journal* 8 (2): 74-78, 2009; doi: 10.3923/ppj.2009.74.78)

### **The Use of Antioxidants and Microelements for Controlling Damping-Off Caused by *Rhizoctonia solani* and Charcoal Rot Caused by *Macrophomina phaseoliana* on Sunflower**

K.M. Abd El-Hai, M.A. El-Metwally, S.M. El-Baz and A.M. Zeid

Seed soaking method or foliar spray of antioxidants (citric acid and salicylic acid at 10 mM) and microelements (manganese and zinc at 2 g L<sup>-1</sup>) were tested to control of the damping-off and charcoal rot diseases of sunflower (varieties Sakha 53 and Giza 102). Field treatments in two different localities i.e., Tag El-Ezz, Dakahlia province and El-Serow, Damietta province were carried out. The high frequency isolated fungi (*M. phaseolina* and *R. solani*) presented in Tag El-Ezz location. On the other hand, *M. phaseolina* was isolated at a high frequency compared with *R. solani* in both locations. Sakha 53 was highly susceptible compared with Giza 102 when artificially infected with both *M. phaseolina* and *R. solani*. Laboratory results showed that salicylic acid alone or in combination with citric acid completely inhibited the linear growth of both pathogens i.e., *M. phaseoliana* and *R. solani* *in vitro*. A positive correlation between the concentrations of Rizolex-T 50 and its effect on the fungal growth were recorded. The dose of 3 g L<sup>-1</sup> prevented the growth of *R. solani* linear growth *in vitro*. The greenhouse results revealed that Giza 102 variety was highly susceptible to the infection by *R. solani*. *M. phaseoliana* showed severe symptoms in both sunflower varieties. On contrary, Giza 102 variety was tolerant to damping-off and charcoal rot diseases than Sakha 53 under field conditions. The application of Rizolex-T 50 followed by citric acid showed a highest percentage of healthy plants followed by the combination of citric acid and salicylic acid. The application of manganese combined with zinc was more effective than the microelements alone. All treatments of antioxidants and microelements

significantly reduced the incidence of charcoal rot disease. On the other hand, no significant differences between Rizolex-T 50 and salicylic acid treatments was shown. Sakha 53 variety gave the highest values of plant height and number of leaves plant<sup>-1</sup> while Giza 102 recorded the highest values of stem diameter and flower head diameter. The application of citric acid combined with salicylic acid maximized the plant height followed by the mixture of manganese and zinc. Manganese treatment followed by the mixture of citric acid and salicylic acid then zinc were the most effective in increasing the number of leaves plant<sup>-1</sup>. While, Rizolex-T 50 had no significant effect on plant height and number of leaves plant<sup>-1</sup>. Microelements were more effective than antioxidants on enhancing the stem and flower head diameters. The combination between manganese and zinc followed by Rizolex-T 50 recorded the maximum values of the stem and flower head diameters. Giza 102 variety recorded the highest values of 100 seeds weight, total phenols, photosynthetic pigments and the percentage of seed oil when the above applications were carried out. Sakha 53 variety showed the highest plant yield under the above treatment. The mixtures of citric and salicylic acids were highly effective in increasing plant yield. The highest values of photosynthetic pigments were shown in salicylic acid treatment followed by Rizolex-T 50. Total phenols content was highest due to Rizolex-T 50 application followed by salicylic acid. Except zinc, seed oil concentration increased significantly in both antioxidants and microelements treatments on oil concentration. (*Plant Pathology Journal* 8 (3): 79-89, 2009; *doi*: 10.3923/ppj.2009.79.89)

### **Seed-Borne Pathogens of Faba Bean in Egypt: Detection and Pathogenicity**

M.A. Elwakil, I.M. El-Refai, O.A. Awadallah, M.A. El-Metwally and M.S. Mohammed

This study was undertaken to study the seed-borne fungi of faba bean that attack the plants and reduce their yield in Egypt. The results provide a database for further study to control the pathogens. Twenty-six seed samples representing six faba bean cultivars collected from different parts of Egypt were used in this investigation. The blotter and deep-freezing methods were used. Surface- and non-surface-sterilized faba bean seeds were tested to detect and isolate the associated seed-borne fungi. The following 20 fungal species belonging to 13 genera were observed and identified: *Aspergillus flavus* (Link ex. Gray), *Aspergillus niger* (Van Tieghem), *Aspergillus ochraceus* (Wilhelm), *Penicillium digitatum* (Pers.:Fr.) Sacc., *Penicillium italicum* (Wehmer), *Alternaria alternata* (Fr.) Keissler, *Botrytis faba* (Sardina), *Cephalosporium* sp.,

*Cladosporium cladosporioides* (Frensen. de Vries), *Epicoccum nigrum* (Link), *Fusarium oxysporum* (Schlechtendahl), *Fusarium semitectum* (Berkeley and Ravenel), *Fusarium solani* (Mart.) Sacc., *Fusarium verticillioides* (*moniliforme*) (Sheld), *Rhizoctonia solani* (Kühn), *Rhizopus stolonifer* (Ehr. ex Fr.), *Stemphylium globuliferum* (Vestergr.) E.G. Simmons), *Trichothecium roseum* (Pers.) Link, *Verticillium dahliae* (Ehrenp) Vuill. The blotter method yielded a greater number of fungi than the deep-freezing method on both surface and non-surface sterilized seeds, but the deep-freezing method was better for slow-growing fungi. The pathogenicity test revealed that the most commonly isolated fungi from pre- and post-emergence damping-off and stunted seedlings were *F. verticillioides*, *R. solani*, *Cephalosporium* sp. and *V. dahliae*. These fungi significantly reduced the photosynthetic pigments in faba bean leaves. *Fusarium verticillioides* caused the greatest reduction in chlorophyll content (A, B and total chlorophyll). *Fusarium oxysporum* and *V. dahliae* significantly reduced carotenoid content. *R. solani* significantly reduced total phenols content when compared with the other tested fungi. (*Plant Pathology Journal* 8 (3): 90-97, 2009; doi: 10.3923/ppj.2009.90.97)

### **Isolation and Evaluation of Indigenous Fungal and Bacterial Isolates as Potential Bioagents Against Broomrape (*Orobanche cernua*) in Jordan**

S.J. Goussous, K.M. Hameed and I. Saadoun

Isolation of microorganisms antagonistic to *Orobanche* was attempted using *Orobanche* plants and rhizosphere soil of *Orobanche*-infected crops in Jordan. Six fungi (*Cephalosporium* sp., *Cylindrocladium* sp., *Epicoccum* sp., *Fusarium* sp., *F. oxysporum* and *F. solani*) were isolated. Of these, isolates that belonged to the *Fusarium* genus were the most common (more than 80% of isolates). Pathogenicity tests of these isolates on *O. cernua* stems indicated that *Cylindrocladium* sp., *Fusarium* sp., *F. oxysporum* and *F. solani* were most effective. These organisms caused total necrosis of inoculated stem tissues. *Epicoccum* sp. caused moderate damage (60% severity); while *Cephalosporium* sp. was least effective causing only localized necrosis. Only *Cylindrocladium* and *Fusarium* sp. isolates infected *Orobanche* inflorescence, causing maceration of these tissues as well as total destruction of seeds. Several bacterial isolates, including *Pseudomonas* and *Actinomyces* sp., were also recovered from soil sampled from different agricultural fields. These bacteria were found to be pathogenic in varying degrees to *Orobanche* stems and inflorescence. A previously isolated *Streptomyces* sp., R9, was also assessed in this study for its

ability to inhibit *O. cernua* seed germination. Results showed a germination rate of 1-1.4% and 2.9-3.8% for R9 crude and lyophilized culture filtrates, respectively. In comparison, seed germination rates were 33.8-42.2% and 6.2-9.1% for water and culture medium controls, respectively. This study indicates that a wide range of microorganisms could be employed as antagonists to *Orobanche*. Their use as potential bioherbicides to control *Orobanche* appears promising. (*Plant Pathology Journal* 8 (3): 98-105, 2009; doi: 10.3923/ppj.2009.98.105)

### **Isolation and Evaluation of Indigenous Fungal and Bacterial Isolates as Potential Bioagents Against Broomrape (*Orobanche cernua*) in Jordan**

S.F. Hwang, H.U. Ahmed, B.D. Gossen, H.R. Kutcher, S.A. Brandt, S.E. Strelkov, K.F. Chang and G.D. Turnbull

Impact of long-term crop rotations on populations of soilborne pathogens in the genera *Fusarium*, *Pythium* and *Rhizoctonia* and on canola seedling establishment and development was evaluated under controlled conditions. Soil samples were collected from two crop rotation experiments conducted at two sites in Saskatchewan, Canada. A part of the 2006-soil sample of each rotation was sterilized to compare canola seedling growth with and without soilborne pathogens. With 2007-soil, the sterilization treatment was replaced with a fungicide seed treatment (Apron Maxx) to assess the potential to reduce seedling diseases. Populations of *Fusarium*, *Pythium* and *Rhizoctonia* sp. were estimated in the soil of each rotation using dilution plating onto selective media for each fungus. Higher seedling emergence and increased growth of canola were obtained in the sterilized soil or due to seed treatment. *Fusarium* was the predominant genus followed by *Pythium* and *Rhizoctonia* sp. in the soil of both sites. This study suggests that diverse crop in the rotation may reduce the populations of *Fusarium*, *Pythium* and *Rhizoctonia* in the soil and may contribute to improve the overall growth of canola. (*Plant Pathology Journal* 8 (3): 106-112, 2009; doi: 10.3923/ppj.2009.106.112)

### **The Occurrence of Aflatoxins in Maize and Distribution of Mycotoxin-Producing Fungi in Eastern Kenya**

J.W. Muthomi, L.N. Njenga, J.K. Gathumbi and G.N. Chemining'wa

Aflatoxin poisoning resulting from consumption of contaminated maize has continued to recur in a yearly pattern in Eastern Kenya. The largest mycotoxin-

poisoning epidemic in the last decade was reported in Kenya in 2004. Therefore, this study was carried out to determine the occurrence and levels of mycotoxin-producing fungi and aflatoxin B<sub>1</sub> in maize and soils from Eastern Kenya. Maize, soils and mill dust samples were collected from farmers and traders in Machakos to determine the incidence of mycotoxin-producing fungi and aflatoxins during the 2007 harvest season. Fungal isolation was done by plating on agar medium, while aflatoxin B<sub>1</sub> was determined by ELISA. The most frequently isolated fungi were *Fusarium* and *Aspergillus* species and the *Aspergillus* species identified were *A. flavus*, *A. niger*, *A. terreus* and *A. versicolor*. *Aspergillus flavus* was frequently isolated from mill dust and soils from under the stores. Aflatoxin levels of up to 160 µg kg<sup>-1</sup> were detected in samples from areas with high *A. flavus* isolation and in whole maize than in semi-processed grain. Most mill dust samples were contaminated with aflatoxin up to 80 µg kg<sup>-1</sup>. The results indicate that *A. flavus* is the main producer of aflatoxins in maize Machakos and high inoculum levels of the fungus are present in soils, near stores and maize mills. Therefore, management of aflatoxin poisoning should include reduction of *A. flavus* inoculum in farms and storage environment. (*Plant Pathology Journal* 8 (3): 113-119, 2009; doi: 10.3923/ppj.2009.113.119)

### **Causal Agents of Root Rot and the Effect of Vesicular-Arbuscular Mycorrhizal Fungi in Seedlings of *Rhodiola rosea* in Alberta, Canada**

S.F. Hwang, H.U. Ahmed, K. Ampong-Nyarko, S.E. Strelkov, R.J. Howard and G.D. Turnbull

*Rhodiola* (*Rhodiola rosea*) is a plant with adaptogenic properties and is suitable for cultivation in Alberta, Canada. Disease surveys indicated the occurrence of root rots in rhodiola plantations in the Province. A total of 74 fungal isolates were associated with discoloration and rotting in the crown and root regions of the plants. Among these, 15 isolates were identified as *Fusarium* sp., three as *Pythium* sp. and eight as *Rhizoctonia* sp. This is the first report of root rot in rhodiola in Alberta, Canada. These soil-borne pathogens are the potential threat to the quality and quantity of rhodiola production. Experiments were conducted to determine the effect of vesicular-arbuscular mycorrhizal fungi and these soil pathogens on rhodiola growth and development under greenhouse conditions. Overall results indicated that *Fusarium*, *Pythium* and *Rhizoctonia* sp. are all capable of reducing rhodiola biomass. However, biomass was significantly higher when vesicular-arbuscular mycorrhizal fungi were applied in conjunction with these pathogens or in non-inoculated controls. This suggests that vesicular-arbuscular

mycorrhizal fungi could be used as a management tool for the control of seedling root rot diseases of rhodiola. (*Plant Pathology Journal* 8 (3): 120-126, 2009; *doi*: 10.3923/ppj.2009.120.126)

### **Expression of Phenylpropanoid Pathway Genes in Chickpea Defense Against Race 3 of *Ascochyta rabiei***

H.R. Kavousi, H. Marashi, J. Mozafari and A.R. Bagheri

The fungal disease, ascochyta blight, caused by *Ascochyta rabiei* is a major yield limiting factor of chickpea (*Cicer arietinum* L.) around the world. Expression analysis of genes induced in general defense response can provide clues to elucidate major defense mechanisms against pathogen infection in chickpea plants. The role of key phenylpropanoid pathway enzymes response to *Ascochyta rabiei* in chickpea was studied under greenhouse conditions using a reverse transcription and semi-quantitative polymerase chain reaction (SQ-PCR). Transcript accumulation of four genes encoding phenylalanine ammonia-lyase (PAL), chalcon synthase (CHS), isoflavone reductase (IFR) and Flavanone 3-Hydroxylase (F3H) induced in response to race 3 of *A. rabiei* was compared in resistant and susceptible genotypes. Results obtained in this study showed that in resistant genotype all 4 phenylpropanoid pathway genes: PAL, CHS, IFR and F3H were rapidly up regulated 6 h after inoculation with race 3 of *A. rabiei*. However, transcripts of PAL and IFR genes were rapidly accumulated in both resistant and susceptible cultivars. Therefore, induction of key enzymes of phenylpropanoid pathway appeared to be an important defense mechanism of chickpea plants against *A. rabiei*. (*Plant Pathology Journal* 8 (3): 127-132, 2009; *doi*: 10.3923/ppj.2009.127.132)

### **The Use of Bread Yeast as a Biocontrol Agent for Controlling Seed-Borne Fungi of Faba Bean**

M.A. Elwakil, O.A. Awadallah, I.M. El-Refai, M.A. El-Metwally and M.S. Mohammed

Present objective was to study this phenomenon on the common seed-borne fungi of faba bean in soil amended with composted organic wastes and infested with the most commonly isolated fungi from *Vicia faba* seed. *In vitro* studies showed that the yeast was effective in reducing the linear growth of *Cephalosporium* sp., *F. verticillioides*, *F. oxysporum*, *F. solani*, *R. solani* and *V. dahliae*. Pre- and post-emergence damping-off caused by *Cephalosporium* sp., *F. verticillioides*,

*F. oxysporum*, *F. solani*, *R. solani* and *V. dahliae* was reduced significantly when seeds of faba bean were coated with a water suspension ( $10^9$  cfu mL<sup>-1</sup>) of the yeast before sowing in soil supplemented with compost type (1) (prepared by Mansoura manufacturer with organic waste from city garbage) or type (2) (consisted of 1 ton of horticultural waste and 100 kg sheep manure). Soil was artificially infested with the fungi isolated from faba bean seeds. The above treatment significantly increased plant growth parameters including height, shoot and root length, number of branches/plant, number of pods/plant, pod weight/plant, fresh weight and dry weight. Photosynthetic pigments (chlorophyll A, chlorophyll B and carotenoids) were also increased by the treatments. Total phenols content in the treated plant leaves was higher than in the control plants. (*Plant Pathology Journal* 8 (4): 133-143, 2009 doi: 10.3923/ppj.2009.133.143)

### ***In vitro* Assay of Factors Affecting the Growth of Pathogens Associated with Diseases on Dragon Fruit (*Hylocereus* spp.) in Peninsular Malaysia**

M. Masyahit, K. Sijam, Y. Awang and M.G.M. Satar

Knowing the unfavorable environment for the growth of a pathogen can be utilized as the basic information in developing appropriate strategies to prevent disease occurrence on dragon fruit. Several environmental factors including temperature, pH and salinity, as well as biotic factor including three antagonistic bacteria species, namely *Bukholderia cepacia*, *B. multivorans* and *Pseudomonas aeruginosa* against *Bipolaris* sp., *Colletotrichum gloeosporioides*, *Botryosphaeria* sp. and *Monilinia* sp., were investigated. Mycelial growth of all tested fungi was constantly inhibited by a temperature of 35°C, while a temperature of 25°C was quite suitable for their growth. A temperature of 30°C was favorable for the growth of *Colletotrichum gloeosporioides*. Under different pH condition, the growth of tested fungi was mostly inhibited by extreme pH of 4 and 10. The salinity assay showed that *Monilinia* sp. was not affected by all treatments among tested fungi. Only concentration 100 ppm could reduce the growth of *Bipolaris* sp., though its inhibition statistically affected on 4 and 6 Days after Incubation (DAI). Meanwhile, the *in vitro* examination of antagonistic bacteria resulted in *Bukholderia multivorans* which was highly effective in inhibiting the growth of examined fungi, except *Monilinia* sp., which was more significantly influenced by *B. multivorans* and *B. cepacia*. The proper combination of environmental modification may be useful for the growth of crop in the field as well as the storage life of the fruit at postharvest preservation. (*Plant Pathology Journal* 8 (4): 144-151, 2009; doi: 10.3923/ppj.2009.144.151)

## **Sensitivity of *Penicillium digitatum* and *P. italicum* to Imazalil and Thiabendazole in Morocco**

H. Boubaker, B. Saadi, E.H. Boudyach and A.A. Benaoumar

Green and blue molds (caused by *Penicillium digitatum* and *P. italicum*, respectively) are the main postharvest diseases of citrus fruits in Morocco. Following packing houses reports of reduced efficacy of fungicides used to control these diseases, a survey was conducted during 2005-2006 packing season to characterize, both qualitatively and quantitatively, the resistance of *P. digitatum* and *P. italicum* to imazalil (IMZ) and thiabendazole (TBZ). Isolates of *P. digitatum* and *P. italicum* were obtained from decayed citrus fruits collected from commercial citrus packing houses located in the Souss-Massa-Draa (SMD), South of Morocco and were evaluated *in-vitro* for their sensitivity to IMZ and TBZ. Of the 290 *P. digitatum* isolates, 19% (55/290) were resistant to IMZ and 37% (107/290) were resistant to TBZ tested at discriminatory concentrations of 0.1 or 20  $\mu\text{g mL}^{-1}$ , respectively. In contrast, only 2.5 (5/204) and 21% (44/204) of *P. italicum* isolates collected from packing houses were resistant to IMZ and TBZ, respectively. No resistance to TBZ and IMZ were detected in *Penicillium* sp., isolates collected from a citrus orchard which has no known history of fungicide use. The proportion of collected isolates that were resistant to both fungicides was 1.5% for *P. italicum* and 10% for *P. digitatum*. The mean  $\text{EC}_{50}$  values for *in vitro* inhibition of mycelial growth of the *P. digitatum* resistant-isolates were between 0.81 and 0.98  $\mu\text{g mL}^{-1}$  for IMZ, whereas those of TBZ were between 39.23 and 50.84  $\mu\text{g mL}^{-1}$ . The mean  $\text{EC}_{50}$  values for *P. italicum* resistant-isolates ranged from 0.53 to 0.61  $\mu\text{g mL}^{-1}$  for IMZ and from 52.97 to 59.92  $\mu\text{g mL}^{-1}$  for TBZ, whereas the mean  $\text{EC}_{50}$  values for orchard collected isolates were 0.04  $\mu\text{g mL}^{-1}$  for IMZ and 0.16  $\mu\text{g mL}^{-1}$  for TBZ. The data will provide a baseline for monitoring resistance to IMZ and TBZ in populations of *P. digitatum* and *P. italicum* in the SMD commercial citrus packing houses in the future. (*Plant Pathology Journal* 8 (4): 152-158, 2009; doi: 10.3923/ppj.2009.152.158)

## **Integrated Management of *Meloidogyne incognita* Infecting Soybean by Certain Organic Amendments, *Bacillus thuringiensis*, *Trichoderma harzianum* and Oxamyl with Reference to NPK and Total Chlorophyll Statuus**

A.G. El-Sherif and Amona F.A. Ismail



The integrated control of *M. incognita* infecting soybean cv. Giza 21 using camel manure, dried leaf powder of marigold, *Trichoderma harzianum* 100% filtrate, *Bacillus thuringiensis* singly or in combination with oxamyl under greenhouse condition ( $22\pm 3^{\circ}\text{C}$ ) indicated that the concomitant treatments obviously gave better results than single ones did. Moreover, *B. thuringiensis* plus oxamyl at half dose each surpassed all other tested materials in percentage increase of total plant fresh and shoot dry weights (99 and 88 %), respectively, followed by *T. harzianum* filtrate plus oxamyl (95 and 61%) in this respect. On the other hand, pots received *T. harzianum* filtrate plus oxamyl ranked first in suppressing final nematode population (90%), root galling (65%) and egg mass numbers (62.79%), followed by B.t. plus oxamyl, marigold powder + oxamyl and then camel manure + oxamyl, where their reduction percentage values amounted to 88.7, 62.5 and 61%; 88.5, 59.87 and 58.9% and 67, 50.5 and 52.97%, respectively. Of the tested singly applications, *T. harzianum* filtrate showed the best results in improving plant growth and suppressing nematode development criteria, followed by B.t., whereas, marigold powder and camel manure achieved the lowest values in this respect comparing to nematode alone. Regarding the N, P and K concentrations in leaves of soybean plants inoculated with *M. incognita* treated with certain organic matters or fungal filtrate or bacterium alone or mixed with oxamyl, they were obviously enhanced by all tested materials whereas the opposite results was recorded for total chlorophyll content comparing to nematode alone. (*Plant Pathology Journal* 8 (4): 159-164, 2009; doi: 10.3923/ppj.2009.159.164)

### ***Puccinia pimpinellae*, a New Pathogen on Anise Seed in Egypt**

K.M. Ghoneem, M.A. Elwakil and A. El-Sadek Ismail

Routine seed health inspection of anise seeds showed *Puccinia pimpinellae* to be a commonly observed fungus on seed samples collected from different locations and the commercial markets of Egypt. Symptoms were shown as black discolorations on seeds. Masses of uredio- and teliospores of the fungus were visually seen. In some samples, a seed washing technique was essential to inspect for the presence of the fungal spores. This is the first report of *Puccinia pimpinellae* as a seed-borne pathogen of anise in Egypt. (*Plant Pathology Journal* 8 (4): 165-169, 2009; doi: 10.3923/ppj.2009.165.169)

## **Analysis of Gene Effects and Inheritance of Resistance to *Fusarium moniliforme* Ear Rot in Maize**

T. Deng-Feng, Z. Fan, Z. Zhi-Ming, W. Yuan-Qi, Y. Ke-Cheng, R. Ting-Zhao, Y. Guang-Sheng and P. Guang-Tang

The inheritance and gene effects on resistance to *F. moniliforme* in maize were investigated using 6 relevant generations of a cross (R15×Ye 478) including 2 parents, F<sub>1</sub>, F<sub>2</sub>, BC<sub>P1</sub> and BC<sub>P2</sub> by the mixed major gene plus poly-genes in genetic model of quantitative traits. The female parent R15, developed by the Maize Research Institute in the Sichuan Agricultural University is highly resistant to ear rot. Ye 478 is an inbred line with a high combining ability, but it is susceptible to many diseases. The frequency distribution of disease severity in segregating populations showed characteristics of a mixed normal distribution, which indicated the inheritance of resistance followed major genes plus poly-genes model. Twenty-four genetic models were established, which could be classified into five types: one major gene, two major genes, polygene, one major gene plus polygene and two major genes plus polygene. Results showed the genetic model E-3 was the most suitable model for the trait and the resistance was controlled by two additive major genes plus additive-dominance polygene. Finally, the results also revealed that agronomic traits investigated such as spike length, spike width, spike rows and kernel depth, etc. had less correlation to resistance to *F. moniliforme* maize ear rot, which showed that resistance to maize ear rot was mainly controlled by genetic factors and indirect agronomic traits can not be used as a selection index in breeding maize varieties for resistant to *F. moniliforme* ear rot. (*Asian Journal of Plant Pathology* 3 (1): 1-7, 2009 doi: 10.3923/ajppaj.2009.1.7)

## ***In vitro* Selection: A Novel Source of Resistance to *Sclerotinia* Stem Rot in Canola (*Brassica napus* L.)**

F. Chamandoosti

In this research, effect of different concentrations of oxalic acid (OAA) (0, 0.02, 0.2, 2 and 20 mM L<sup>-1</sup>) were studied on callusing and organogenesis of canola in order to possibility producing resistant canola plant to pathogen fungus *Sclerotinia sclerotiorum* (Lib.) de Bary by this pressure selection. 0-0.2 mM L<sup>-1</sup> OAA had a positive effect on above reactions so that the mean diameter of calli and the mean number of regenerated roots and shoots on hypocotyl explants, also the mean number of explants that produced callus and de novo organs were

increased. These results showed that the addition of OAA (0.0-20 mM L<sup>-1</sup>) in canola culture media (*in vitro* selection technique) lead to produce resistant canola plants to *Sclerotinia sclerotiorum* (Lib.) de Bary. (*Asian Journal of Plant Pathology* 3 (1): 8-13, 2009 doi: 10.3923/ajppaj.2009.8.13)

### **Identification and Pathogenicity of *Fusarium* Species Associated with Root Rot and Stem Rot of *Dendrobium***

Z. Latiffah, M.Z. Nur Hayati, S. Baharuddin and Z.Maziah

Three *Fusarium* species, *F. oxysporum*, *F. proliferatum* and *F. solani* were isolated from root and stem rot of *Dendrobium* orchid. Their pathogenicity on *Dendrobium* was studied and the inoculation test showed that the three *Fusarium* species were pathogenic, causing root and stem rot on the orchid. Molecular characterization using PCR-RFLP of ITS+5.8S regions showed that the isolates from the same species produced similar patterns and UPGMA cluster analysis of PCR-RFLP of ITS+5.8S clearly grouped *F. oxysporum*, *F. proliferatum* and *F. solani* into separate clusters. The present study showed that three *Fusarium* species, *F. oxysporum*, *F. proliferatum* and *F. solani* were associated with root and stem rot of *Dendrobium* orchid. (*Asian Journal of Plant Pathology* 3 (1): 14-21, 2009 doi: 10.3923/ajppaj.2009.14.21)

### **Detection of Presumptive Mycoparasites in Soil Placed on Host-Colonized Agar Plates in Riyadh Region, Saudi Arabia**

Y.Y. Molan

The presumptive mycoparasites *Trichoderma* sp., *Pythium* sp., *Gliocladium* sp. and *Verticillium* sp. were detected in 42 (51.2%), 2 (2.5%), 23 (28.4%) and 1 (1.2%) of a total 81 soils, respectively, when soil samples were placed on sectors of potato dextrose agar colonized by the appropriate host fungi. Most (81) of the soils in the study contained three or more mycoparasities, but the frequency of detection on replicate host sectors suggested that *Trichoderma* sp. and *Gliocladium* sp. were the more abundant species in all soils in which they occurred. The type of host fungus markedly influenced the efficiency of detection of the different mycoparasities: *Fusarium* sp. was most efficient for *Trichoderma* sp. and *Gliocladium* sp. and *Rhizoctonia solani* for *Trichoderma* sp. Only a single host was suitable for consistent detection of each of the mycoparasite species. (*Asian Journal of Plant Pathology*, 3 (1): 22-26, 2009 doi: 10.3923/ajppaj.2009.22.26)

## **Identification and Coat Protein Nucleotide Sequence of Turnip Mosaic Potyvirus from *Eruca sativa* in Saudi Arabia**

M.A. AL-Saleh, I.M. Al-Shahwan, O.A. Abdalla and M.A. Amer

The goal behind this study was the identification, biological and molecular characterization of a virus infecting garden rocket (*Eruca sativa* Mill) and producing severe symptoms on it in Riyadh region, Saudi Arabia. Mosaic, stunting and deformation were observed on field grown garden rocket plants. Mechanical inoculation of twelve plant species with sap prepared from infected garden rocket plants resulted in infection of four of them. These were *E. sativa* Mill, *Raphanus sativus* L., *Brassica rapa* L. and *Chenopodium ammaranticolor* Cost and Reyn L. Symptoms on the first three plant species were systemic mosaic, stunting and deformation, whereas symptoms on *C. ammaranticolor* were local lesions. *Brevicoryne brassicae* L., transmitted the virus to *E. sativa* in a non-persistent manner. Electronmicroscopic examination of carbon-coated grids prepared by the leaf dip method revealed flexuous virus particles typical of potyviruses. Enzyme linked immunosorbent assay indicated that samples collected from symptomatic *E. sativa* plants in both Riyadh and Qassim regions were infected with turnip mosaic potyvirus (TuMV). Positive results were also obtained using a Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method to detect and identify TuMV from nucleic acid extracts of the symptomatic garden rocket plants collected from that area, using a specific oligonucleotide primer for detection of TuMV-CP. Nucleic Acid Spot Hybridization Assay (NASH) using DIG labeled cDNA probe showed high levels of hybridization signal, whereas no hybridization was observed with uninfected tissues. The nucleotide sequence of the CP gene of the Saudi Arabian isolate of TuMV detected in garden rocket and tentatively denoted (TuMV-SA-Ro) was determined to be composed of 862 nucleotides in length. (*Asian Journal of Plant Pathology* 3 (2): 27-38, 2009 doi: 10.3923/ajppaj.2009.27.38)

## **Semi-Selective Culture Medium for *Xanthomonas axonopodis* pv. *malvacearum* Detection in Cotton Seeds (*Gossypium hirsutum* L.)**

Cleci Dezordi, Antonio Carlos Maringoni, José Otavio Machado Menten and Renata Cassia Camara

The cotton disease known as angular leaf spot, caused by *Xanthomonas axonopodis* pv. *malvacearum* (Xam) has been causing cotton losses in several

producing regions around the world. Xam is transmitted by seeds, which may be infected both externally and internally. Infected seeds constitute the main long-distance dissemination mode of the pathogen. In view of this, the use of healthy seeds is a must. To accomplish that, detection methodologies for the bacteria must be developed be used in seed health analysis laboratories. This study aimed to develop a semi-selective medium for Xam detection in cotton seeds. The semi-selective culture medium was named MSSXAN and it was consisted of peptone (5.0 g), beef extract (3 g), sucrose (5 g), soluble starch (10 g), agar (15 g), CaCl<sub>2</sub> (0.25 g), Tween 80 (10 mL), distilled water (1,000 mL), crystal violet solution at 1% (150 µL), cephalixin (50 mg<sup>1\*</sup>), methyl thyophanate (10 mg<sup>\*</sup>) and chlorothalonil (10 mg<sup>\*</sup>) - \*added after culture medium autoclaving. This MSSXAN medium shows low repressiveness to Xam and it be used for isolation of this bacteria in cotton seeds health analysis. (*Asian Journal of Plant Pathology* 3 (2): 39-49, 2009 **doi:** 10.3923/ajppaj.2009.39.49)