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Screening of endophytic endospore-forming bacteria associated to *Theobroma cacao* and antagonistic to *Moniliophthora roreri* in Quinindé, Esmeraldas, Ecuador

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The aim of this work was the screening of endophytic endospore-forming bacteria associated to *Theobroma cacao* (cv. Criollo type National), with antagonistic activity against *Moniliophthora roreri* (Cif.) H.C Evans *et al.* in Quinindé, Esmeraldas, Ecuador. Isolation of the bacteria was carried out from sections of pod tissues and flower cushion, without apparent symptoms of the disease. Subsequently, the bacterial isolates were evaluated *in vitro* to identify their antagonistic capacity against *M. roreri* as well as their chitinolytic activity. In addition, the production of volatile metabolites with effect on mycelial growth and sporulation of the fungus was determined in selected isolates. Finally, promising isolates were identified molecularly from the sequencing of the gene encoding 16S rRNA. Forty-five isolates of endospore-forming endophytic bacteria were obtained from tissue sampled mainly from the endocarp. Isolates 9-2, 24, 29-2, 33, 45-1, 51-2, 53-4 and 72 showed high antagonistic capacity *in vitro* against *M. roreri* and chitinolytic activity. In addition, it was found that bacterial isolates 9-2, 24, 33 and 45-1 produced volatile metabolites that inhibited mycelial growth and sporulation of *M. roreri* by more than 60%. The sequencing showed that isolates 9-2, 24, 29-2 and 45-1, 51-2, 53-4 presented a similarity of 99% with *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus mycoides* and *Bacillus cereus*, respectively; while isolates 33 and 72 did not produce an optimal consensus sequence.

***Trichoderma* antagonism against gray mold (*Botrytis cinerea*) in blackberry crop in Costa Rica**

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The gray mold caused by *Botrytis cinerea* is the most important disease affecting blackberry crop in Costa Rica and measures to control the disease are necessary to improve yield production and quality. *In vitro* assays (dual culture tests) were conducted to investigate the antagonistic effects of 4 native *Trichoderma* spp. isolates retrieved from berries carpoplane of healthy plants against *B. cinerea* collected from diseased fruit. After 8 days of incubation, the following parameters were assessed: 1) direct antagonistic effect and classification according to Bell's scale, 2) effect of volatile metabolites measured according to the % inhibition of radial growth, and 3) mycoparasitism that was evaluated according to a five grades scale. The 4 *Trichoderma* isolates showed an efficient antagonistic effect against *B. cinerea* and were identified as grade II (Bell's classification). The isolates also showed a high percent of radial growth

inhibition (>70%) indicating that mycelia growth of *B. cinerea* was inhibited by production of volatile metabolites. Three *Trichoderma* isolates were allocated as grade III according to the scale to evaluate mycoparasitism, and one isolate was identified as grade II showing that all isolates were efficient mycoparasites. The *Trichoderma* species of the isolates is being determined. In conclusion, all native *Trichoderma* isolates exhibited antagonistic effects against *B. cinerea* and their potential to control gray mold under field conditions must be determined.

Selection of promising bean genotypes resistant to BGYMV with the molecular marker bgm-1

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The common bean (*Phaseolus vulgaris* L.) is one of the most important grain legumes for human consumption. In Cuba, there are different factors that limit its production, among which the bean golden mosaic disease, caused mainly by the begomovirus Bean golden yellow mosaic virus (BGYMV). Genetic improvement for resistance to this virus is one of the most effective strategies for disease control. The objective of this work was the identification of the resistance gene *bgm-1* from the codominant SCAR marker S2 (Blair *et al.*, 2006) in commercial varieties and advanced lines. In addition, the severity of the viral symptoms was evaluated to each genotype in flowering stage according to the scale proposed by CIAT (1991). DNA extraction was performed on 83 genotypes. From the PCR analysis with the S2 primers, it was possible to identify 30 genotypes with presence of the *bgm-1* gene in recessive state, which confers the resistance to the BGYMV. Of these, 24 had no symptoms caused by begomovirus. The CUL 156, Delicias-364 and Velazco Largo genotypes had the highest incidence of symptoms, with severe symptoms. The results of this work constitute a great contribution to the common bean improvement program in Cuba, because much of the genotypes analyzed are materials from the bean breeding programs of the region.

Current situation of *Xanthomonas* spp and *Phaseolus vulgaris* L. pathosystem in the west region of Cuba

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The common blight bacteria (CBB) disease is caused by the plant pathogenic bacteria *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) and *Xanthomonas citri* subsp. *fuscans* (*Xcf*), which could cause yield losses around 10 and 40% in common bean. The characterization of *Xanthomonas* spp. isolates from different locations producing common bean in the Mayabeque province was the main goal of this work. Samples of leaves and pods from naturally infected plants were collected and processed to isolate and identify the pathogenic bacteria. Isolation was performed in culture media (YDCA and XCP1) and the pathogen was detected by Polymerase Chain Reaction (PCR) with specific primers. Biological tests were used for the nutritional characterization as well as the rep-PCR technique and the sequencing of Housekeeping genes for the molecular characterization of the identified isolates. In addition, the pathogenicity of these isolates was evaluated in the susceptible cultivar *Bat-304*, as well as in resistant and susceptible genotypes from Canada. A DNA fragment was obtained using PCR and the size of the fragment corresponded to the size reported already for these *Xanthomonas* species. The biochemical-physiological characterization revealed a high nutritional similarity between the isolates obtained. However, the molecular and pathogenic characterization showed a high variability among the isolates identified. Therefore, these outcomes constitute an evidence of the diversity of *Xanthomonas* spp. isolates that circulate into the common beans production areas; in addition, they indicate the need to deepen the knowledge of the molecular determinants related with this variability.

Temporal distribution of plant-parasitic nematodes on select turfgrass sites in Alabama

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Total revenue from turfgrass in the United States has been estimated at over 60 billion dollars, and the state of Alabama has the third highest number of sod farms in the United States. Turfgrass represented in this study included golf courses, turf farms with representatives of different sod types, and experimental plots with different turfgrass varieties. Plant-parasitic nematodes can cause unthriftiness and unsightly turf which is undesirable in certain turfgrass situations. These nematodes parasitize turf roots in the upper 4" of the soil and add stress to turf that is mowed at a low height, is under water, compaction, or other abiotic stresses. It is important to know which plant-parasitic nematodes are present as some are more damaging than others. Previous research has shown that these nematodes can go through multiple generations during a year. The specific nematode, the number of generations per year and the length of the specific nematode life cycle are important information to help manage these pests in turfgrass. Six turfgrass sites were sampled throughout the growing season for presence and quantification of plant-parasitic nematodes. Sites were chosen for the geographical location in Alabama and also for the type of turfgrass host present. Plant-parasitic nematodes were extracted using sucrose density centrifugation, identified, and quantified. Five genera of plant-parasitic nematodes were found, with one of them never recovered at damaging levels; however, four were frequently found at levels capable of causing plant damage. The average length of each nematode's life cycle varied with the time of the year and the specific nematode.

Identification of resistance genes to Begomovirus and Tospovirus in tomato genotypes in Cuba

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The viral diseases caused by the genera Begomovirus and Tospovirus, in the last decades have caused important losses in crops of economic interest (tomato, potato pepper, tobacco) in Cuba. In the face of such damages, genetic improvement programs have been implemented in Cuba to develop resistant varieties that allow better management and control of diseases. The Ty.1, Ty.2 and Ty.3 genes for resistance to TYLCV and SW5 for TSWV are the genes that have been most used in tomato breeding programs. In this study, twelve tomato genotypes conserved in the germplasm of the National Institute of Agricultural Sciences (INCA) were analyzed. DNA was extracted according to the protocol proposed by Permingeat, 1998. Gene amplification was carried out by PCR and the corresponding specific primers were used. The presence of the alleles was identified to the Ty.3 resistance gene in three of the genotypes analyzed (genotypes 201, 205, 210); the allele corresponding to the Ty.2 gene was detected in genotypes 201, 203, 205. The allele corresponding to resistance gene SW5 was only observed in genotypes 205 and 207. The results indicate the presence of resistant genes to TYLCV and TSWV in a pyramid combination in genotype 205 and its use is suggested, as well as the rest of the genotypes with the presence of some resistant genes studied in genetic improvement programs. The importance of using molecular markers to achieve progress in obtaining varieties with resistance to the diseases with the greatest impact on production is confirmed.

Plant-growth promoting rhizobacteria: A potential management strategy for *Meloidogyne* spp. on turfgrass

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Meloidogyne spp., root-knot nematodes, are among the most economically important plant-parasitic nematodes in turfgrass in the United States. Only a few nematicides are available or efficacious for plant-parasitic nematodes in turfgrass in the U.S., and recent work in our lab has demonstrated the potential for microbial control of root-knot nematodes in field crops. The objectives of this study are to evaluate the ability of 104 plant growth-promoting rhizobacteria (PGPR) strains isolated from grasses in Alabama for their mortality of *M. incognita* in-vitro, and their ability to manage plant-parasitic nematodes in the greenhouse setting. Strains were evaluated in-vitro in 96-well plates, with mortality from each strain compared to non-treated and chemical nematicide, fluopyram, after 24, 48, and 96 hours. Of these 104 strains, 51% are *Bacillus* spp., 25% are other genera, and 24% are still unknown as identification is still ongoing. In-vitro mortality ranged from 2% to 93%, with an average mortality of 22%. Eleven strains were selected for further screening in a turfgrass system. These include 7 *Bacillus* spp., 1 *Comamonas* sp., 1 *Gordonia* sp., 1 unknown species, and Blend 20, a blend of three *Bacillus* strains previously found to enhance growth and drought tolerance in grasses. Greenhouse evaluations showed 7 of the 11 strains significantly reduced *M. incognita* juvenile numbers, and 4 strains significantly increasing root dry weight.

Fluazaindoline effects on *Belonolaimus* spp. nematodes in turf

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Belonolaimus spp., the sting nematode, is an economically important plant-parasitic nematode on turf grass. In these studies, our objectives were to evaluate the potential of fluazaindoline to reduce population densities of the sting nematode and enhance turf quality in greenhouse, microplot, and field trials. All trials included fluazaindoline at four rates compared to fluopyram and an untreated control and were established in a randomized complete block design with five to six replicates and each test was repeated. Data were analyzed using SAS 9.4 PROC GLIMMIX and LS means separated using Tukey-Kramer ($P \geq 0.05$). In the greenhouse trials, *Belonolaimus* spp. populations were reduced at all rates of fluazaindoline and fluopyram as compared to the untreated control. Microplot trials also indicated a 93% reduction in *Belonolaimus* spp. populations with the application of the nematicides compared to the control ($P \geq 0.05$) from the June application through the final September sampling. *Belonolaimus* spp. population density was well above threshold at 27 per 100 cm³ of soil in the field trial at nematicide application. Over the season, the nematicides kept the *Belonolaimus* spp. levels reduced by 80% compared to the control ($P \geq 0.05$). Visually the NDVI green ratings in the microplots and field trials were improved ($P \geq 0.05$) in the higher rates of fluazaindoline and fluopyram indicating an improvement in plant health. These results demonstrated that fluazaindoline can reduce *Belonolaimus* spp. population density in the greenhouse, microplot, and field conditions, and increased turf health.

Morphological characterization of *Boeremia* spp. causal agent of black spot disease in common bean (*Phaseolus vulgaris* L.) in Colombia

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In Colombia, many common bean varieties are susceptible to different diseases including black spot disease also known as Ascochyta blight. The disease was reported in Colombia several years ago, in recent years high economic losses have been reported. This disease is one of the main diseases that affects common bean in cool producing regions. The typical symptoms include black lesions exhibiting in concentric rings. The disease can be present in leaves, branches, nodes and pods. Severe attack can lead to premature defoliation and plant collapse. The main reported species are: *Boeremia exigua*, *B. noackiana*, *B. diversispora* and *Stagonosporopsis*

hortensis. To determine what species are causing black spot in Colombia, 50 isolates were collected from the main producing areas located in the departments of Antioquia, Boyacá, Cauca, Cundinamarca, Huila, Nariño and Santander. *Boeremia* species identification was based on the conidia morphology and mycelial growth rate. Forty-eight isolates were identified as *B. noackiana* (96%) and two isolates were identified as *B. exigua* (4%). For both species, high variability was observed in the conidia size, color and shape. Aerial mycelia and growth rate was also variable. Conidia were elliptical and cylindrical in shape and hyaline to light brown, aseptate or 1-septate. For *B. exigua* the size of the conidia ranged from $4-12 \times 2.5-3.5 \mu\text{m}$. For *B. noackiana* the size of conidia ranged from $4-7 \mu\text{m} \times 2.5-3.5 \mu\text{m}$. The diameter of the colonies of *B. exigua* and *B. noackiana* ranged from 4.5 to 12 and from 4 to 12 cm, respectively. Genetic and pathogenicity analysis are being conducted.

Cassava mosaic diseases in the Huila province, Angola

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Cassava mosaic disease (CMD) has a complex viral etiology, and has been associated with different virus species, mainly in the genus Begomovirus. The objective of this study was to evaluate the epidemiological situation of the disease in the province of Huila, determine the viral species associated and propose management tactics based on the analysis of the risk factors of the disease and the evaluation of cultivars present in the country. Twenty production fields were evaluated and surveys were conducted in grower fields in the months of June in 2016, February and June in 2017 and February in 2018. The symptoms of the disease were present in 100% of the fields analyzed without significantly differed for periods in both locations. The density of the whitefly (*Bemisia tabaci* spp) in Caluquembe, during June 2016, was highest while in Kuvango it was lower. It showed that temperature and humidity had the greatest influence in both locations, together with rainfall and altitude. The age of the cuttings at the time of the transplant and the absence of cultural practices during the presence of vectors, old fields among other factors were factors of high significance for the risk of the disease in both locations. In symptomatic plants the African Cassava Mosaic Virus (ACMV) was detected without detecting other associated begomovirus species. The behavior of 19 foreign and 21 national cultivars were evaluated and cultivars were identified with better behavior in the field and presence of alleles related to the CMD2 gene that could be introduced in breeding programs for resistance to the disease.

New weeds as '*Candidatus* Phytoplasma sp. host in Cuba

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Phytoplasmas have emerged as one of the main factors limiting the production of several important crops. They are cell wall-free and phloem-limited bacteria. They are transmitted mainly by sap-feeding insect vectors of the Hemiptera order. Weeds play a significant role in the epidemiology of diseases because of their capacity to house the causal agents and function as a refuge for their vectors. In Cuba, the presence of phytoplasma in weeds associated with the tomato and pepper crops has not been reported. Therefore, we intended to detect '*Candidatus* Phytoplasma sp. in weeds in suburban tomato and pepper producing areas in Cuba. Surveys were conducted in 2017 and 2018 in suburban tomato and pepper producing areas in the locality of Guanabacoa, Havana province. Thirty samples were collected from symptomless weeds including *Parthenium hysterophorus* L., *Senna alata* (L.) Roxb., *Ocimum sanctum* L., *Bidens alba* (L.) DC., *Portulaca oleracea* L., *Euphorbia heterophylla* L., *Amaranthus albus* L., *Digitaria sanguinalis* L. (Scop.). Twenty samples were also taken from symptomatic tomato and pepper plants. The DNA was extracted and analyzed by a nested PCR using universal primers. Phytoplasmas were detected in the tomato and pepper plants and in 16 weed samples corresponding to: *Parthenium hysterophorus* L., *Senna alata* (L.) Roxb., *Bidens alba* (L.) DC., *Portulaca oleracea* L., *Amaranthus albus* L. To our knowledge, these results are the first reports for Cuba of these weeds as phytoplasma hosts. These results are an evidence of an increase of the host range of this pathogen in Cuba.

Serological, molecular, and microscopic virus detection in *Canna edulis* Ker. in Nariño, Colombia

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The achira plant (*Canna edulis* Ker.) is a cultivated species used for handcrafted food products and starch production in Colombia. The area devoted to cultivation of the crop is ~800 ha. In the department of Nariño, a disease outbreak of suspect viral etiology was observed, referred to by the local farmers as 'streak virus'. To determine the identity of the suspect viral pathogen a study was carried out at the University of Nariño. The symptoms observed in the affected achira plants included foliar yellow mosaic, mottling and chlorotic streaking, and rib discoloration. The samples were analyzed using several approaches, including polymerase chain reaction (PCR) and reverse transcriptase (RT)-PCR amplification, DNA sequencing, serological analysis, and transmission electron microscopy (TEM). Based on TEM, leaves contained lamellar cytoplasmic inclusions similar to those attributed to other well-studied potyviruses. The results of the PCR amplification using potyvirus-specific primers, and sequencing of the expected size product (fragment of cDNA), the suspect virus shared 98% homology with *Sugarcane mosaic virus* (SCMV). This is the first report of the detection of a SCMV-like virus in symptomatic achira plants grown in Nariño, Colombia.