P49 INFLUENCE OF APPLICATION TIME ON SURVIVAL, ESTABLISHMENT AND ABILITY OF *CLONOSTACHYS ROSEA* TO CONTROL *BOTRYTIS CINEREA* ON ROSES

Marcelo A. B. Morandi, Liliana P. V. Mattos, Elen R. Santos

Embrapa Environment, CP 69, 13820-000, Jaguariúna, SP, Brazil; e-mail: mmorandi@cnpma.embrapa.br.

The effect of sunlight on the establishment of *Clonostachys rosea* on phylloplane has received little attention. We investigated the influence of application time (9:00, 12:00, 15:00, and 18:00 h) and the length of exposure to natural sunlight (0, 0.5, 1, 2, 4, and 8 h) on survival, establishment, and ability of the antagonist to suppress *Botrytis cinerea* sporulation on rose leaves. The experiments were carried out in a climate-controlled greenhouse (experiment 1) and in a plastic-covered greenhouse without climatic control (experiment 2).

The germination of the conidia correlated negatively with application time from 9:00 to 18:00 h, and the recovery of viable conidia from leaves reduced exponentially with length of exposure to sunlight (cfu=42,46*exp[-0,2224*t]; R^2=0,94). The conidia germination was significantly increased in the treatment kept on shadow. However, germination incidence was inversely proportional to the application time on all treatments, independently of exposure to sunlight. These finds indicate that other factors beside solar radiation influenced the conidia germination. The relative humidity (RH) within three and six hours following inoculation correlated positively with germination, independently of sunlight exposure. These results are in accordance with previous findings showing that RH was the main factor that influenced the establishment of *C. rosea* on rose debris in a commercial plastic-covered greenhouse.

Colonization of tissues by *C. rosea* was significantly reduced (40 to 50%) as exposure to sunlight increased. Despite drastic effects of exposure to sunlight on *C. rosea*, suppression of *B. cinerea* sporulation was only marginally affected (suppression of 94.5 to 100% and 65 to 93% at experiment 1 and experiment 2, respectively).

Climatic conditions on the time of application and the following hours are crucial for *C. rosea* establishment in host tissues. Exposure of conidia to direct sunlight, low RH, and high temperature on the phylloplane for several hours reduced the efficiency *C. rosea* in colonizing tissues and partially affect its ability to suppress *B. cinerea*. These findings support the importance in studying ecological attributes of biocontrol agents in order to avoid failure in their efficiency in the field.

Data from our studies indicate that *C. rosea* is highly sensitive to solar radiation and that the application time of the antagonist to the phylloplane, can influence its establishment. Given the importance of these microclimatic factors to *C. rosea* survival and ability to control the pathogen, it would appear to be advantageous to schedule the antagonist application to the end of the day, during periods of low sunlight and higher humidity.

Supported by FAPESP (2002/02243-3 and 2006/02247-0)

P71 CONTROL OF CITRUS BLACK SPOT (*GUIGNARDIA CITRICARPA*) BY BIOLOGICAL CONTROL AGENTS AND OTHER ALTERNATIVE PRODUCTS

Wagner Bettiol, Eduardo R. A. Bernardo

Laboratory of Environmental Microbiology, Embrápa Environment, CP 69, 13820-000 Jaguariúna, São Paulo, Brazil, e-mail: bettiol@cnpma.embrapa.br.

Citrus black spot (CBS) has been responsible for substantial damage in citrus, in different countries. In Brazil, the disease occurs in several municipalities in the State of São Paulo, in an area that is highly representative of the state's citriculture. Black spot control basically relies on the use of protective or systemic fungicides, applied at 28-day intervals. The objective of this work was to evaluate the effects of biocontrol agents (*Bacillus subtilis* and *Trichoderma* sp.) and other alternative products (cow milk and biofertilizer) to control CBS in organic and conventional systems.

In the first experiment, the following treatments were done: *B. subtilis* (10^7 and 10^8 UFC/ml); autoclaved Milhocina (0.5%) + Molasses (0.5%); *Trichoderma* sp. (10^6 conidia/ml); cow milk (5%) and Microgeo® (commercial biofertilizer currently used by citrus organic growers). All treatments, except Microgeo®, were sprayed at scheduled intervals (0, 28, 56, 84, 112, 140, and 168 days) from December 8, 2004 (bloom period) to August 28, 2005 (fruit harvest). The Microgeo® treatment was sprayed along all year at a monthly interval. The experiment was conducted in a completely randomized blocks design with 5 treatments and 15 replication plants ('Pera'). The severity of the disease on 50 fruits at harvest stage collected randomly from each replication plant were evaluated by means of a six-category scale, where 1=0.5%, and 6=49% of fruit area with lesions. The percentage of fruits classified at class 1, 2 and 3 to 6 were calculated. The milk and *B. subtilis* (10^8) treatments did not differed significantly from each other and presented the higher percentage of fruits classified at class 1 (26.3% and 19.4%, respectively) and the lower percentage of fruits at class 3 to 6 (29.9% and 35.6%, respectively). The milk and *B. subtilis* treatments were significantly superior to Microgel® treatment (10.9% e 50.8%, respectively for fruits at class 1, 2 and 3 to 6).

In the second experiment, in a 'Valência', on conventional system orchard, different doses (0, 2.5, 5, 7.5, and 10% v/v) of a biofertilizer (produced by aerobic fermentation of a mixture of molasses, compost cattle manure, earthworm humus, yeast and water) were sprayed at the same dates as the first experiment and compared with a standard fungicide treatment. The % of fruits classified as 1 and 2 were 4.4, 8.0, 5.8, 6.6, 4.9 and 12.8, respectively for the treatments 0, 2.5, 5.0, 7.5 and 10% of biofertilizer and fungicide. The percentage of fruits classified as 5 and 6, for the same treatments were 38.4, 29.5, 27.8, 28.4, 28.0, and 19.4. The results show the potential of biofertilizer, milk and *B. subtilis* as an alternative for citrus black spot control, especially in organic agriculture.

P61 **ENDOPHYTIC BACTERIA FOR BIOCONTROL OF COFFEE LEAF RUST (*HEMILEIA VASTATRIX*)**

Wagner Bettiol[1], Harllen Sandro A. Silva[2], Itamar Soares de Melo[1], César R.F. Terrasan[1], João Paulo L. Tozzi[1], Flávia Vieira Nunes[1]

[1]Laboratory of Environmental Microbiology – Embrapa Environment, CP 69, 13820-000 Jaguariúna, São Paulo, Brazil, e-mail: bettiol@cnpma.embrapa.br; [2]Instituto Biológico, CP 70, 13001-970 Campinas, São Paulo, Brazil, e-mail: harllen@biologico.sp.gov.br

Endophytic microorganisms are used for biological control of plant diseases and for enhanced agronomic plant characteristics. Several mechanisms may control the suppression of plant pathogens, either directly by antibiosis and competition, or indirectly by induction of plant resistance response. The objective of this work was to select endophytic bacteria strains from coffee leaves (F) and stems (G) with biocontrol potential against coffee leaf rust (*Hemileia vastatrix*). Two hundred thirty two endophytic bacterial strains were evaluated in coffee leaf discs. Bacterial suspensions were applied on leaf discs, 72 and 24 hours before, after and simultaneously with the pathogen. As results, nine bacterial strains (116G, 123G, 36F, 137G, 14F, 109G, 115G, 3F, and 119G) showed to be effective in reducing the rust development. These selected bacterial strains were evaluated in coffee seedlings (*Coffea arabica* 'Mundo Novo'). The bacterial suspensions were sprayed to the foliage 72 and 24 hours before, after, and simultaneously with the pathogen. The best control levels were obtained when the biocontrol agents were applied 72 hours before the pathogen. Four endophytic strains - 119G, 3F, 115G, and 109G - were effective in controlling the coffee leaf rust (89%, 84%, 69%, and 66%, respectively).

The activity of enzymes (peroxidase, lipoxygenase, and phenilalanine ammonia-liase) was assessed in relation to the control of *H. vastatrix* in leaf coffee seedlings seven days after the spray of four bacterial strains (119G, 3F, 115G, and 109G). It was observed that the inoculation of the strains 3F and 119G increased the peroxidase activity in leaves of coffee seedlings and significantly reduced the number of rust lesions per leaf. The other enzymes were not affected. The detection of peroxidase activity in leaves without the presence of the antagonists and pathogen proves the induction of systemic resistance, but probably there are other mechanisms of action. The isolates were identified based on cell membrane fatty acid contents, analyzed in a gas chromatograph, using microbial identification software (MIDI Sherlock TSBA Library version 5.0, Microbial ID, USA), as 3F=*Bacillus coagulans*, 115G=*Microbacterium testaceum*, and 119G=*Cedecea davisae*.

This project was supported by FAPESP (99/09177-1).