

## COST OFFICE - SHORT-TERM SCIENTIFIC MISSIONS (STSM)

### SCIENTIFIC REPORT

Beneficiary/Applicant: Ph.D. Milicevic Tihomir, Faculty of Agriculture, University of Zagreb

Host: Conte Elisa, Plant Pathology Research Institute (Rome, Italy)

Period of STSM: from 13. December 2006. to 23. December 2006.

Place: Rome (Italy)

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**STSM Topic: Phytopathological analysis of the most important pathogens on strawberry**

#### **Purpose of the visit**

The improvement of basic methods of isolation and determination of plant pathogenic fungi on strawberries, first of all species from genus *Phytophthora* and other fungi which are the major phytosanitary problem in the nurseries during the production of healthy strawberry seedlings and during cultivation.

#### **Description of work carried out during the visit**

13.12. 2006.

Arrival in Rome. Accommodation in the hotel.

14.12. 2006.

Meeting with the host Ph.D. Elisa Conte. I was introduced with the Plant Pathology Research Institute, its scientific work, departments or sections and the head of each department. All the scientific work on the Institute is distributed through six departments: General plant pathology and physiopathology, Epidemiology and resistance to diseases, Virus and virus-like diseases, Cryptogamic diseases, Plant protection and Forest pathology. At the end of the day the head of Institute Ph.D. Marina Barba and I have decided that it is for the best, accordingly to my suggested work program and the purpose of my visit, to incorporate my STSM in the work of department «Cryptogamic diseases» (with Ph.D. Alessandra Belisario on the head) that is specialized for *Phytophthora* species on varies host plants, including strawberry.

15.12. 2006.

Meeting with Ph.D. Alessandra Belisario. We have arranged the purpose of my STSM – Methods of isolation and determination of the most important *Phytophthora* species. Besides *Phytophthora* species I have also had an opportunity to observe the isolation and determination of *Pyrenochaeta* species. However due to lower importance of *Pyrenochaeta* in my scientific work, I have mostly based my work on *Phytophthora* species. As the larger number of scientists in the Institute work on different methods of isolation and determination of *Phytophthora*

species, I could acquire a good insight in this field of study. As a main method, we have chosen «baiting» isolation technique of *Phytophthora* spp.

### **1. Description of “baiting” isolation technique of *Phytophthora* spp. from the infected soil**

Samples of infected soil around a plant are taken 15 cm around the plant and from 15 cm depth. Samples must not be too wet or too dry and are placed in a noted PVC bags, each sample separately. In a laboratory the soil samples are arranged in 200 ml plastic cups. Approximately 2 cm of soil should be put in a cup and poured with distilled water. After that, the leaves of plants that are used as a decoy for *Phytophthora* (*Rhododendron* or *Azalea*, *Quercus*, etc.) are put on a water surface. We have used *Rhododendron* spp. as a decoy in our research, two leaves per cup. Incubation period of 5-7 days at temperature 20-22 °C should produce brown infection areas on the leaves if the *Phytophthora* sp. was present in the soil. Afterwards a leaf is cut to slices (approx. 2-3 mm) and is put on selective media prepared for growth of *Phytophthora* species, and for incubation in dark for 4-5 days at temperature 22 °C. Among several existing recipes for preparation of selective media we used the following:

Recipe for selective media for *Phytophthora* species (Vetraino et al., 2001)

15g/l Malt extract

20g/l Agar Oxoid No.3

996 ml distilled water

Autoclave at 121 °C for 15 minutes.

Antibiotics including piramicin (10mg/l), ampicillin (250 mg/l), rifampicin (10 mg/l), benomyl (15 mg/l) and hymexazol (50 mg/l) were dissolved in distilled water (2 ml) and ethanol (2 ml).

Antibiotics are added when the media reaches 45-50 °C. This way prepared media can be conserved for 4-5 days at 4°C.

### **Determination and detection of *Phytophthora* species**

We start the determination after the appearance of *Phytophthora* mycelia. Determination is made based on morphological characteristics of sporulation organs - zoosporangiophors with zoosporangia and oogonia with oospores.

To produce zoosporangia, special solution for sporangial production has to be made. We have used the following (Ribeiro, 1978):

0,4 g - Ca (NO<sub>3</sub>)<sub>2</sub>

0,15 g - MgSO<sub>4</sub> x 7H<sub>2</sub>O

0,15 g - KH<sub>2</sub>PO<sub>4</sub>

0,06 g - KCL

1000 ml - distilled water

Into the solution, poured into Petri plates, small disks of mycelia are put and are incubated on 12 – 20 °C. Sporangia are usually produced in 2 – 3 days.

Morphological criteria for determination of *Phytophthora* spp.

<b>Morphological characteristic of asexual and sexual fructification structures</b>
Zoosporangiophore branching
Shape of zoosporangia
Size (length and width) of zoosporangia (in $\mu\text{m}$ )
Presence of papillate
Presence of pedicel and its length (in $\mu\text{m}$ )
Shape of antheridia (amphigynous or paragynous)
Size of antheridia (in $\mu\text{m}$ )
Size of oogonia (in $\mu\text{m}$ )
Size of oospores (in $\mu\text{m}$ )

### **Description of the main results obtained**

Based on described methods of isolation and determination on tested samples of infected soil, we have isolated and determined the following species of *Phytophthora*: *P. nicotianae*, *P. cinnamomi*, *P. cactorum* and *P. hedraiaandra*. With the approval of head of Department, the isolated *Phytophthora* species (*P. nicotianae*, *P. cinnamomi*, *P. cactorum* and *P. hedraiaandra*) were taken to be brought to my home Department in Croatia as a standard sample for the *Phytophthora* collection. Also, during determination I have learned to differentiate the *Pythium* species from *Phytophthora* species, which is important since *Pythium* species are often found during isolation of *Phytophthora* species in infected soil. The work described above took place between 15.12- 20.12.2006.

21.12.2006.

I was in the Institute's mycological collection of plant pathogenic fungi and got acquainted with the preservation methods in this kind of collection (isolates stored on a leaning PDA media covered with paraffin oil). I have also got the approval to take some isolates of fungi that are pathogens on strawberries for my future scientific researches in Croatia (*Phytophthora cinnamomi*, *Phytophthora nicotianae*, *Phytophthora cactorum*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Verticillium albo-atrum* and *Verticillium dahliae*). Unfortunately, I haven't been able to acquire other species of the genera *Phytophthora* (*P. fragariae*), *Colletotrichum* (*C. fragariae*) and *Rhizoctonia* (*R. fragariae* and *R. solani*), which are specific pathogens on strawberries, because they were not available in the mycological collection at that period.

22.12.2006.

I have viewed the experimental facilities of the Institute (greenhouses etc.) where the pathogenic organisms on host plants are tested and the rest of the day spent in an extensive library of the Institute reading books and some new journals with studies about pathogens on strawberries. I have copied a larger number of the newest articles from scientific journals which my host department doesn't receive.

23.12.  
Return to Croatia.

### **Future collaboration with host institution**

We have arranged the possibility for future collaboration and cooperation through some scientific projects in phytopathology that could be of bilateral benefit for our institutions and countries, both Italy and Croatia.

### **Other Comments:**

Special thanks to the Management Committee of Cost Action for assigned STSM, to host Ph.D. Elisa Conte and the whole Plant Pathology Research Institute, especially to head of Institute Marina Barba.

In Zagreb, 15.01.2007.

Applicant  
Ph.D. Tihomir Milicevic