Monitoring of Soft Fruit Mother Plantings Aimed at Control of *Phytophthora fragariae*, Causal Agent of Root Rot

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Summary

Phytophthora fragariae was first detected in the Republic of Serbia in 2002, and it has been included in A2 quarantine list of damaging organisms since 2003. The project titled 'Monitoring of soft fruit mother plantings aimed at the control of *Phytophthora fragariae*, causal agent of root rot' was realized over 2004 – 2005 aiming at determination of population rate of the pathogen and the control of raspberry planting material. Over that period, the total 388 samples were tested. Collected samples were analyzed by PCR. The presence of *Phytophthora fragariae* was detected in 156 samples.

Key words

Phytophthora fragariae, raspberry mother planting, planting material, PCR

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Introduction

Raspberry is one of economically most significant fruit cultures in the Republic of Serbia. The total raspberry grown area in our country amounts to 15,000 ha, whereas raspberry production ranges from 75,000 – 93,000 t (Milenković *et al*., 2005). Over the 90's of the XX century, a sudden and pronounced incidence of dieback of commercial raspberry plantings was recorded. Within the project titled 'Integral fruit and grapevine protection' (BTR.5.04.0527.B) a number of commercial raspberry plantings with symptoms of dieback in different localities of Serbia was observed during 2002. The samples were collected and forwarded to the Scottish Crop Research Institute, Dundee, Scotland aiming at testing for the presence of *P. fragariae*. Several methods of detection were applied: nested PCR, 'baiting' test and isolation of the pathogen on the selective 'French bean' medium. The pathogen was detected by application of PCR in nine out of 14 samples forwarded to SCRI. The analysis took two days. After five weeks, only two out of potential 200 isolates were obtained by isolation on the selective medium. The 'bait' test showed presence of *P. fragariae* in the samples after six weeks.

Phytophthora species are considered primary causal agents of root rot in raspberry in all productive regions of the world, and, as pathogens of these cultures, were first detected in 1937 (Wilcox, 1989). Eight different species were isolated from raspberry root: *P. fragariae*, *P. megasperma*, *P. erytroseptica, P. cactorum, P. citricola, P. cryptogea, P. drechsleri* and *P. cambivora* (Duncan, 1996).

In both EU and EPPO, *P. fragariae* is of A2 pathogen status, with no tolerance (0%) in planting material.

Following the EU and EPPO standards for the control of mother plantations, the project titled 'Monitoring of soft fruit mother plantations aimed at control of *Phytophthora fragariae*, causal agent of root rot' was completed over 2004 – 2005.

The major objectives of the project were testing of registered and unregistered soft fruit mother plantations for the presence of *P. fragariae* by nested PCR, education of owners of mother plantings and preparation of recommendations for the establishment of new mother plantings. The project was realized through the following phases: 1- sampling of the planting material, and 2 - sample analysis.

Material and method

Sampling of planting material

The sampling was carried out in compliance with the current EPPO standards for the testing of raspberry mother plantings for the presence of *P. fragariae* (in spring, when plants are small and drying sections or individual plants are easy to observe). The number of samples is not standardized by EPPO depending on the assessment of person who performs sampling. We adopted the sampling approved by Dr. James Duncan (personal communication). Plants with distinctive *Phytophthora fragariae* symptoms were collected. When symptoms were not obvious, plants of low habitus were collected. Average sample included 10 – 15 plants from 5 m^2 area.

Laboratory analysis

The samples of raspberry planting material were tested by PCR that has not been available on larger scale so far. The method has been employed since 2003 at the laboratory for phytopathology of the Fruit Research Institute Čačak (Both fungus isolation on the nutritive medium and the 'baiting' test, developed by Dr. James Duncan, are considered standard methods by EPPO. At the meeting of EMN in Nancy, France, April 25 – 29, 2004, one of conclusions of the diagnostics panel included proposal that Drs James Duncan and Dave Cook should develop a new protocol for detection of *P. fragariae* in order to make it available to experts).

Pathogen detection started with DNA extraction. Roots were collected from the samples, processed under the running water, and immersed in liquid nitrogen. The extraction was performed by application of DNA extraction buffer (200mM Tris-HCl pH 7.5, 250mM NaCl, 25mM EDTA, 0.5% SDS) and *i*-propanol. The pellet was rinsed with ethanol and re-suspended in distilled water. Upon the extraction, the universal method of DNA purification was applied. Specific primers were utilized for the nested PCR (QIAGEN, Germany). The first cycle involved application of primers ITS4 (White *et al*.,1990) and DC6 (Bonants *et a*l., 1997), used for detection of all *Phytophthora* species, including other species of the *Oomycetes* class. The second cycle included DC1 (Bonnants *et al*., 1997) and DC5 (Bonnants *et al*., 1997) *P.fragariae* specific primers.

PCR products (amplified fragments of DNA) were analyzed by electrophoresis in 5% polyacrylamide gel at 150 V for 2.5 hours, and stained by silver nitrate according to Schumacher *et al.* (1986). As for the first cycle, DNA fragment of 1310 bp, indicating presence of any of *Phytophthora* species, was detected, whereas within the second cycle the specific DNA fragment of 533 bp, distinctive solely for *Phytophthora fragariae* species, was found. The presence of *Phytophthora fragariae* was analyzed in 388 samples.

Results

Aiming at more efficient sample collecting and monitoring of mother plantations, the territory of Serbia was

divided into five regions consisting of 5 – 10 localities each, depending on the number of mother plantations. One-year old and older mother plantations with cvs. Willamette and Meeker, along with those of post-quarantine status were tested. The total 388 samples were tested in the course of the project, 179, 152, and 57 samples collected from old, one-year old, and post-quarantine plantings respectively. Specific DNA fragment of 533 bp molecular mass was detected in 156 samples, i.e. the presence of the pathogen fungus *P. fragariae* (Fig. 1) was detected in the tested samples. The pathogen was detected in 82, 65, and 9 samples collected from old, one-year old, and post-quarantine plantings respectively.

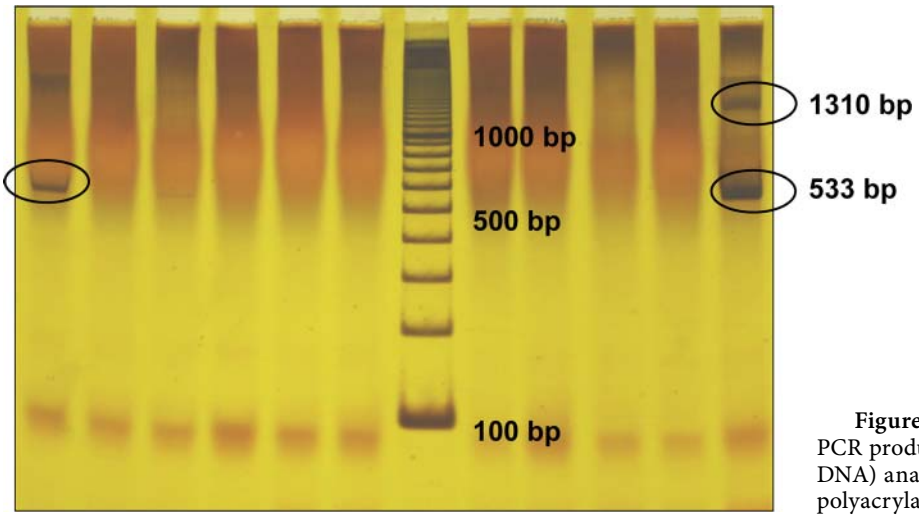

Figure 1.
PCR products (amplified fragments of DNA) analysis by electrophoresis in 5% polyacrylamide gel

Discussion

P. fragariae is widely spread in all raspberry growing regions (Koprivica *et al*., 2003). The reasons that brought about massive spread of the pathogen are trade of infected planting material and inadequate application of basic sanitary, agro-technical and chemical measures in mother and commercial plantations. With regard to the fact that *P. fragariae* belongs to the group of quarantine pathogens, it was essential to come up with the project of control of soft fruit mother plantations, which helped to determine distribution rate of the pathogen in the Republic of Serbia.

The project involved young (one-year old) and old mother plantings, as well as post-quarantine ones (in our country, the Law on Plant Protection orders introduction of post-quarantine for the imported planting material of fruits for two years). Samples collected from the post-quarantine plantings were also among those 156 *P. fragariae* infected samples. The testing of these samples prior to import did not show presence of the pathogen. The reasons for the delayed incidence of the symptoms are as follows:

- during the time of import *P. fragariae* was of the latent status, and the symptoms displayed in the plantations at conditions that favor the development of the pathogen (optimal temperature, water saturated soil);
- transfer of oospore pathogens by watering, soil, shoes, tools, and machines used in different plantings.

Nevertheless, certain samples of the planting material, tested prior to import, showed presence of *P. fragariae* (planting material originating from USA and Poland).

The results of the analysis were forwarded to the Plant Protection Department that ordered eradication of infected mother plantings, in compliance with measures defined by the Law on plant protection aimed at the control of quarantine pathogens. In addition, based on the project results, a Directive was enacted on application of measures aiming at protection of raspberry from the root rot disease caused by *P. fragariae*. The Directive included measures of prevention of incidence and spreading of the root rot disease, notification of any import of the raspberry planting material, measures of control of the planting material marketing and conditions to be fulfilled when establishing new mother plantations.

Conclusion

The results of the project titled 'Monitoring of soft fruit mother plantations aimed at the control of *Phytophthora fragariae*, causal agent of root rot' have ensured determination of the population rate of *P. fragariae* as well as creating distributional map of the pathogen. Infected regions were localized. Removal of the infected mother plantations along with complete removal of pathogen infected commercial plantings was performed. Aiming at education of owners of mother plantations and pro-

ducers, the lectures were conducted on morphological properties and ways of spreading of *P. fragariae*. Instructions were given as regards preventive measures and system of control of the pathogen. Good coordination of phytosanitary officials and laboratories was established, which provided prevention of import of infected planting material.

By appropriate application of the stated measures, spreading of *P. fragariae* has been fully prevented and rigorous control of traded planting material has been ensured. Based on the project results, the Ministry of Agriculture, Forestry and Waterpower Engineering subsequently financed a project titled 'Introduction of certification into propagation of raspberry planting material' in 2005. The final goal of the project has been to make the process of obtaining certified raspberry planting material more rapid. Fruit Research Institute Čačak has been appointed as leader of the project.

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