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THE MOST IMPORTANT PATHOGENS TRANSMITTED BY SUGAR BEET*

ABSTRACT: Pathogenic fungi and viruses transmitted by sugar beet seed represent a complex group of organisms. Detection of these pathogens is an important issue in sugar beet protection. Their identification is a difficult task because the most available methods rely on the growth characteristics, morphological and biochemical criteria. Three domestic and eight foreign sugar beet varieties, from Germany, Italy and Greece were included in the investigation. Seed health testing was performed in laboratory and in field conditions. During the trials, the following methods were used: blotter method, agar plate method and ELISA test for viruses. Seeds were incubated in "Conviron" aparatus at 22°C which is suitable for sporulation of different kind of fungi (light and temperature were adjustable). The appereance of following fungi was noted during incubation: *Pleospora bjoerlingii* (*Phoma betae*), *Fusarium* spp., *Pythium* spp. *Aphanomyces cochlioides* and *Cercospora beticola*. Viruses tested by ELISA test were *beet necrotic yellow vein virus* (BNYVV) and *beet yellows virus* (BYV). Viruses were tested in sugar beet seedlings grown in laboratory conditions and on leaves of individual plants from the field. The disease index was calculated on the basis of intensity of infection of plants for *Cercospora beticola* and *Phoma betae* according to Mc Kinney's formula. Results were presented by graphs, tables and original photos.

KEY WORDS: ELISA test, leasf spot, seed-borne, sugar beet, diseases, viruses

INTRODUCTION

More than 90% of food in the world is produced from the seed. Seed itself often presents the basic source of parasite inoculum. The reasons for giving such attention to pathogens are ever more increasing exchange of seed material and the danger of spreading of new pathogens to those parts of the world where they were not found previously. The exchange of seed material contributed to increased number of seed-borne pathogens. Viruses found in field and vegetable crops only recently gained importance. Economic impor-

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tance of seed-borne parasites led to altered attitude of developed countries towards phytopathology and the seed-borne pathogens. Now, special attention is paid to problems related to quarantine, seed quality determination and chemical protection. Seed certification has become the part of integral management system in plant protection (M i l o š e v i ć, 2001).

Regular control of seed health on the presence of quarantine and economically harmful organisms, in the laboratory and in the quarantine field is necessary due to the increased import of sugar beet seed from different parts of the world. Continual quarantine and post-quarantine supervision is the basis for well developed and stable production as well as for the protection of domestic varieties from uncontrolled import.

Sugar beet seed plays an important role in seed certification scheme. Due to economic importance of this plant species, it is important to be familiar with harmful organisms attacking its seed. The most common sugar beet seed-borne parasites are *Pleospora bjoerlingii* B y f o r d (an. *Phoma betae* B j o e r l i n g) — *Phoma leaf spot* and *damping off*; *Peronospora farinosa* (Fr.) Fr. f. sp. *betae* B y f — *downy mildew*; *Cercospora beticola* S a c c. — *Cercospora leaf spot*; *Ramularia beticola* F a u t r. et L a m b. — *Ramularia leaf spot*; *Uromyces betae* (P e r s.) L e v. — *sugar beet rust*; *Alternaria tenuis* N e e s. — *Alternaria leaf spot*; *Fusarium oxysporum — Fusarium yellows*; *beet necrotic yellow vein virus* (BNYVV); *beet yellows virus* (BYV).

Beet necrotic yellow vein virus is a quarantine parasite listed on the A2 EPPO list No. 160 (OEPP/EPPO, 1988). It is also listed in Rules on health examination of crops and objects for production of seed, seedlings and planting material and health examination of seed, seedling and planting material (*Official register of SRJ*, 66/99). *Beet yellows virus*, *Peronospora farinosa* and *Phoma betae* are also listed as economically significant parasites.

Effective protection of crops from those above mentioned diseases could be achived by complex mesaures in which cultivation practice, the introduction of tolerant varieties into production and the application of fungicides are included (M a r i ć and J e v t i ć, 2001).

MATERIAL AND METHODS

The appearance of quarantine and economically harmful parasites of sugar beet on domestic and foreign varieties was observed. Three domestic and eight foreign sugar beet varieties from Germany, Italy and Greece were included in the investigation. Domestic varieties were marked as S1, S2 and S3; those from Germany as N1, N2, N3 and N4; those from Italy as I1, I2 and from Greece as G1 and G2. Testing was done both in the field and in the laboratory.

Laboratory testing

Seed health testing was done in laboratory conditions in the National laboratory for seed testing as a part of regular testing of samples from domestic

trade and import. Blotter method, method of nutritive media and ELISA test were used as laboratory tests.

Pelleted seed was previously washed in order to remove preparation and initiate the development of seed-borne parasites. The seed for testing on blotter and nutritive media were initially prepared by immersing in 1% NaOCl solution for 5 minutes. After that, the seed was washed (3 times) in sterile water, dried and placed into previously prepared Petri dishes. Ten seeds, from 400 were placed into each Petri dish. The incubation of seed on blotter and potato dextrose agar was done in sterile conditions in Petri dishes for 7 days at 22°C (ISTA Rules, 2002) using alternating light cycle (12h NUV/12h of dark, "Conviron" apparatus). Upon incubation, each seed was observed under a stereo microscope and present pathogens were determined (M a t h u r and K o n g s d a l, 2003).

Viruses were identified using serological method (Enzyme immuno-adsorbtion ELISA test). Samples were tested in two stages. In the first stage, the seed was germinated in sterile boxes and ELISA test was done using obtained sugar beet seedlings. In the second stage, the samples of leaves from field were used. Forty-five seedlings from one variety and 45 leaves from individual plants were tested for both viruses.

Table 1. — ELISA test for BNYVV and BYV

Procedure	Reagent	Time of incubation	Incubation temperature	Number of washing
Incubation of the antybody (NUNC-96 plates)	IgG dilution 1:200 in coating buffer	4 h	37°C	4x
Forming of the antibody-antigen complex	Extraction of samples (seedling) in buffer-relation 1:20	Over night	4°C	5x
Application of the antibody AP-conjugate	AP-conjugate diluted 1:200 in extraction buffer	4 h	37°C	5x
Enzymic assay	Substrate solution	1—2 h	Room temperature	—

Reagents of *beet necrotic yellow vein virus* BNYVV and *beet yellows virus* (BYV) (LOEWE Biochemica GmbH, Germany), Kit Complete consisting of antibody, conjugate, positive and negative control were used. Automatic ELISA reader, Multiskan Ascent at 405 nm was used for reading of Nunc plates with 96 wells.

Field testing

The survey was conducted in 2005 at Rimski Šančevi testing field 1 (quarantine field of National laboratory for seed testing). Chosen varieties were sawn in the first part of April (2005-04-04). Each sample was sawn in two rows using sawing machine for micro trials. Rows were 10 m in length

with 70 cm distance between the rows. Fungicide treatments were not used during vegetation period. Disease intensity was evaluated by exemination of individual plants (each fifth plant) according to scale of 0—9. Disease index was calculated on the basis of the intensity of leaf spot diseases according to Mc Kinney's formula.

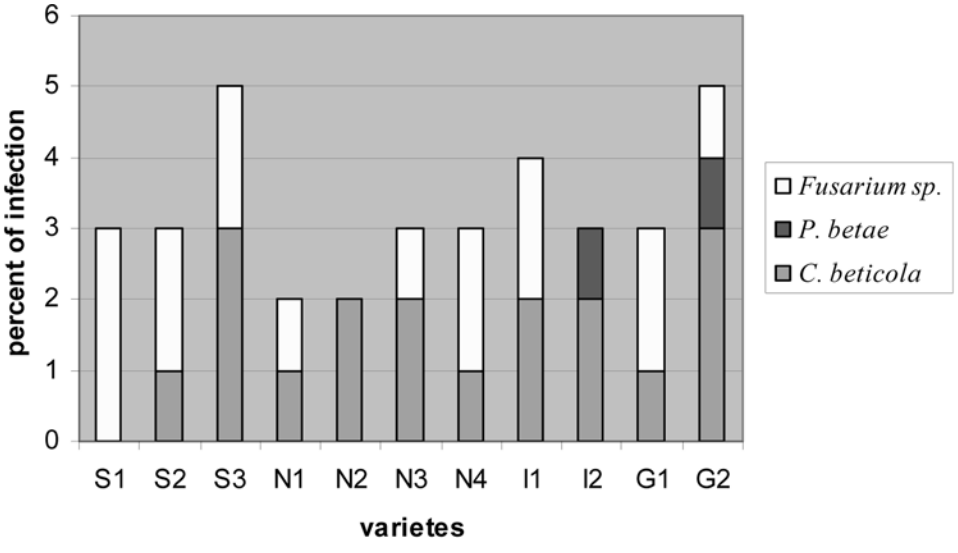
$$I = \frac{\sum(axb)}{kx10} x100$$

I — disease index
a — number of exeminated plants
b — number of categories (0—9)
k — total number of plants
10 — number of categories

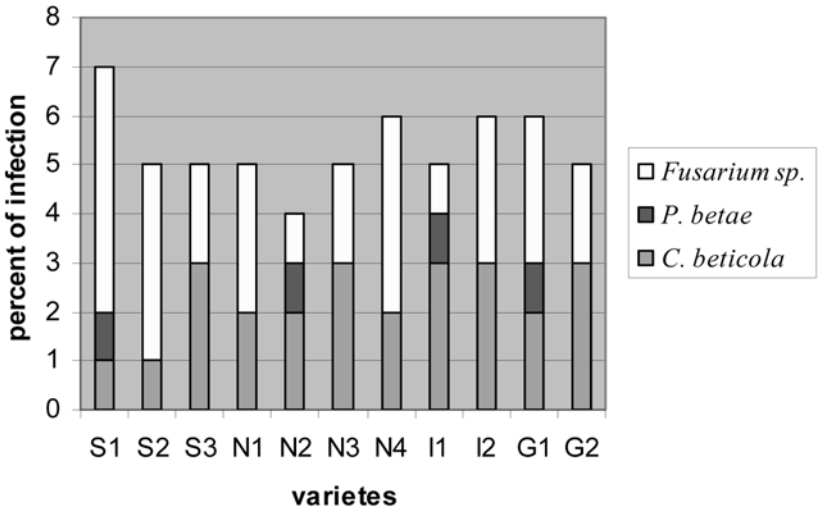
RESULTS

Results of laboratory testing

Results of laboratory testing on blotter and nutritive media are shown in graphs 1 and 2. Total percentage of seed infection ranged from 2—5% on blotter, and from 4—7% on nutritive media. *Aphanomyces cochlioides* and *Pythium* sp. were not found in sugar beet samples.



Graph. 1 — Percentage of sugar beet infection caused by *Fusarium* sp., *Phoma betae* and *Cercospora beticola* on blotter



Graph. 2 — Percentage of sugar beet seed infection caused by *Fusarium* sp., *Phoma betae* and *Cercospora beticola* on nutritive media (PDA)

According to obtained results of ELISA test (Table 2) for sugar beet seedlings, all tested samples were healthy.

Table 2 — Range of absorption values obtained by DAS ELISA test for *beet necrotic yellow vein virus* (BNYV) and *beet yellows virus* (BYV) (from seedlings)

Variety	Beet necrotic yellow vein virus (BNYV)	Beet yellows virus (BYV)
S1	0,074—0,092	0,055—0,111
S2	0,072—0,137	0,041—0,131
S3	0,093—0,134	0,043—0,132
N1	0,076—0,127	0,052—0,121
N2	0,076—0,116	0,051—0,109
N3	0,089—0,133	0,062—0,100
N4	0,083—0,134	0,044—0,110
I1	0,085—0,125	0,055—0,121
I2	0,070—0,122	0,066—0,133
G1	0,072—0,124	0,067—0,119
G2	0,072—0,103	0,058—0,128
Positive control	1,295	0,768
Negative control	0,137	0,118

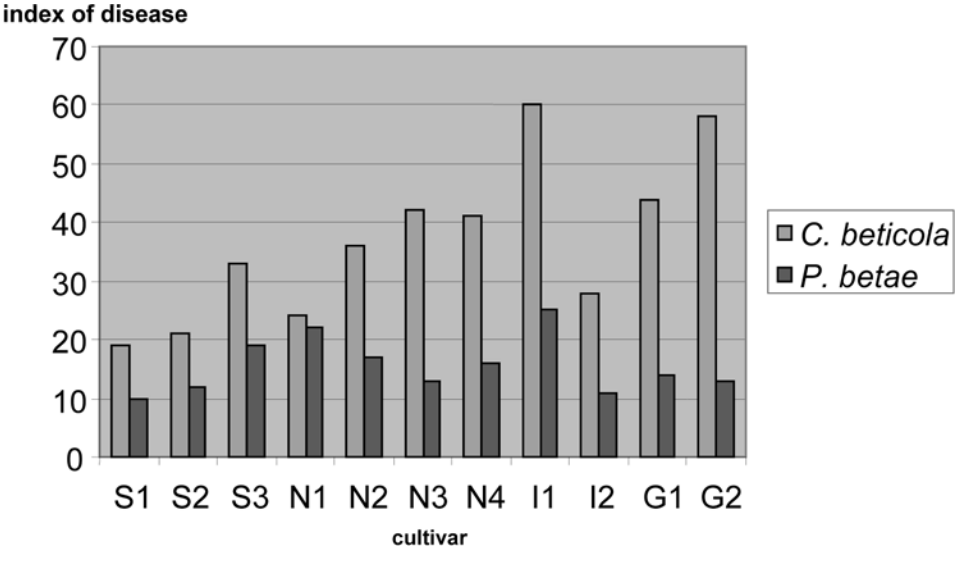
Results of laboratory testing

According to ELISA test (Table 3) results obtained from sugar beet leaves, all tested samples were healthy.

Table 3 — Range of absorption values obtained by DAS ELISA test for *beet necrotic yellow vein virus* (BNYV) and *beet yellows virus* (BYV) (from leaves of individual plants from field)

Variety	beet necrotic yellow vein virus (BNYV) (rhizomania) (BNYV)	beet yellows virus (BYV)
S1	0,081—0,103	0,083—0,103
S2	0,086—0,098	0,089—0,098
S3	0,099—0,112	0,089—0,112
N1	0,101—0,129	0,110—0,129
N2	0,123—0,135	0,111—0,125
N3	0,078—0,141	0,088—0,121
N4	0,085—0,138	0,086—0,118
I1	0,111—0,139	0,101—0,124
I2	0,127—0,130	0,066—0,115
G1	0,106—0,145	0,076—0,125
G2	0,110—0,132	0,100—0,128
Positive control	1,301	0,833
Negative control	0,150	0,121

The high percentage of infection caused by *Cercospora beticola* ranging from 19—60% was noticed based on the results of index of diseases (Graph. 3), while *Phoma betae* was present in much smaller percentage, from 10—25%



Graph 3 — Index of diseases according to Mc Kinney's formula for causal agents of *beet leaf spot*

Symptoms of *sugar beet leaf spot* (*Cercospora beticola* and *Phoma betae*) are shown on figure 1 and 2. *C. beticola* causes appearance of tiny, round, grey spots with dark red tissue zone on the edge of sugar beet leaf (Figure 1). *P. betae* forms large round concentrically zoned spots, often with cracked tissue in the centre (Figure 2).

DISCUSSION

Sugar beet seed health testing (blotter and nutritive media) revealed the presence of parasitic fungi *Phoma betae* and *Cercospora beticola*, and fungi from *Fusarium* genus. Total percentage of infection ranged from 2—5%. From obtained results, it can be seen that *Fusarium* was present on most of the varieties except on N2 and I2. *Phoma betae* was found on two tested varieties: I2 and G2 and the percentage of infection did not exceed 1%. *Cercospora beticola* was not found in domestic variety S1 and the percentage of infection in other varieties ranged from 1—3%.

Nutritive media method revealed the presence of seed parasites in approximately the same percentage as in the method on blotter. Obtained percentage of infection is the result of chemical treatment (pelleted seed) and does not significantly influence disease level on plants in the field (R i c h a r d s o n, 1990).

According to results obtained by ELISA automatic reader at 405 nm, all tested seed samples (seedlings) were negative for both BYV and BNYV, which was also confirmed by comparison of obtained values with positive and negative controls. ELISA test is used as one of serological procedures for pathogen identification. This method is a modern technique in seed health testing (M a c h a d o et al., 2002). High reliability and speed of pathogen detection are major elements in many areas where efficient and precise analyses and results presentation is needed. This method is recommended as an efficient and reliable for determination of latent infections when import consignments are in question. This is especially significant since the possibility of transmission of *beet yellows virus* by seed is mentioned in the literature (N e e r g a a r d, 1979). It is also known that virus of *rhizomania* can be indirectly transmitted by seeds coated with residues of infected beet or soil parts (Š u t i ć, 1995). In

spite of different opinions on seed virus transmission, those in charge of sugar beet import should check the seed on the presence of *beet yellows virus* and *beet necrotic yellow vein virus* in a laboratory and check crops prior to main aphids flight in the field (group of authors, 1980).

ELISA test was also used for samples of individual plants from the field (parts of leaf were taken). Viruses were not found in analyzed samples. Concentration of viruses is usually very unequal and content of viruses and their concentration is higher in older leaves (A g r i o s, 1997). *Beet necrotic yellow vein virus* and *beet yellows virus* are mainly found in phloem, although they can be found in other plant parts too (leaf) (Š u t i ć, 1995). These viruses can be transmitted during vegetation period from overwintered infection sources, such as some weeds, mangel or fodder rape pits, self seeded plants etc. Virus vectors are aphids *Myzus persicae* and *Aphis fabae*. Intensity of infection depends on density of aphid population and sources of infection, especially those close to sugar beet field.

Field trials were observed during vegetation period. Seedling decline was not found in the stage of sugar beet emergence due to fungicide treatment (pelleted seed). Mini-pelleting and pelleting of sugar beet seed is a technological procedure in seed processing when seed is covered with several different micro and macro substances, growth stimulators, fungicides and insecticides (K a w a k a t s u et al., 1998).

Meteorological conditions during vegetation period and especially quantity and schedule of precipitation influenced intensity of appearance of leaf diseases during 2005. No chemical fungicide treatment was used during sugar beet vegetation period. Sugar beet in isolation was sown on the bordering plot (0,1 ha) in the previous year (2003/04) which could be the source of initial parasite inoculum. High percentage of appearance of leaf diseases is the result of combination of above mentioned factors. The greatest values of disease index for *Cercospora beticola* (60%) were found for I1 and G2 varieties, while the smallest index was found for domestic varieties S1 and S2 (20%). *Phoma betae* was found in much lesser percentage, ranging from 10—25%.

Optimal conditions for plant infection are temperature at around 25°C and relative humidity exceeding 95%. By cultivating tolerant variety and using regular agrotechnical measures and chemical protection, the intensity of appearance of sugar beet leaf diseases is decreased. Infected seed could be a potential source of inoculum, but infected residual debris, poor agrotechnical measures and irregular crop rotations make even greater threat. Infected leaves left on field are the most significant source of infection, so regular crop rotation has great influence on disease development.

Regular expert seed health control related to quarantine and economically harmful organisms, done in the acredited laboratories is the basis for high and stable yields besides proper agricultural measures and the application of chemical preparations.

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ЕКОНОМСКИ НАЈЗНАЧАЈНИЈИ ПАРАЗИТИ КОЈИ СЕ ПРЕНОСЕ СЕМЕНОМ ШЕЋЕРНЕ РЕПЕ

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Резиме

Патогене гљиве и вируси који се преносе семеном шећерне репе представљају комплексну групу организама. Испитивање ових патогена је веома значајно у заштити шећерне репе. Њихова идентификација је доста сложена због тога што се многе доступне методе односе на карактеристике пораста, морфолошке и биохемијске особине. У испитивања су биле укључене три домаће и осам страних сорти шећерне репе пореклом из Немачке, Италије и Грчке. Огледи су изведени у лабораторији и у пољу. Здравствено стање семена одређивано је у лабораторијским условима. У току рада коришћене су следеће методе: филтер папир метод, метод хранљиве подлоге и ELISA тест на присуство вируса. Семе је инкубирано у термостатима на 22°C и у „Conviron" апарату где постоји могућност

подешавања светлости и температуре, потребних за спорулацију различитих гљива. Током инкубације праћена је појава следећих паразитних гљива: *Pleospora bjoerlingii* (*Phoma betae*), *Fusarium* spp., *Pythium* spp., *Aphanomyces cochlioides* и *Cercospora beticola*. Вируси су испитивани ELISA тестом и то: Вирус некротичног жутила нерава шећерне репе (Beet necrotic yellow vein virus BNYVV) и Вирус жутице шећерне репе (Beet yellows virus BYV). Вируси су анализирани из поника добијених наклијавањем у лабораторијским условима и из листа појединачних биљака у пољу. На основу интензитета заразе биљака у пољу израчунат је индекс обољења за гљиве *Cercospora beticola* и *Phoma betae* према Mc Kinney-евој формули. Добијени резултати представљени су графиконима, табелама и оригиналним фотографијама.