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## **Preliminary results of the screening of *Euphorbia esula* pathogens collected in Russia**

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### **Introduction:**

Leafy spurge, *Euphorbia esula* L., is a complex of species of Eurasian origin. It is a toxic deep-rooted perennial dominant weed on rangelands and pastures in North America. The first biocontrol project against this weed was started in 1962. Since then, numerous releases of insect and fungal species have been made in order to control leafy spurge in USA and Canada (Julien, 1992; Gassmann and Schroeder, 1995). However, leafy spurge still causes high economic losses in most of the infested area. Obviously, further investigations are necessary to find new biocontrol agents. The present study aimed at field collection and preliminary screening of phytopathogenic fungi promising for biocontrol of leafy spurge.

### **Material and Methods:**

#### *Fungal Strains.*

Diseased plants of *Euphorbia esula* were collected during 1997-1998 in Southern Russia and Siberia. Several tissue fragments were taken from each part of the diseased plants (root, stem, leaves etc.) and were dried and preserved. In total, more than 400 samples of diseased *E. esula* were collected.

Each tissue sample was sterilized with 10% Clorox and placed on selective media. Five types of isolation media were used. Plates were incubated at 28° C for 48-96 hours. Mycelia emerging from the infected tissue was isolated and identified to the genus level (Barnett and Hunter, 1987). Fungal strains that had previously been reported to be plant pathogenic were screened for virulence to *E. esula*. Virulent strains were later identified to the species level. All isolated fungal strains are stored at low temperatures (3-5° C) on agar slants and on colonized toothpicks.

#### *Plant cultivation.*

*Euphorbia esula* plants were grown from roots collected from infestation sites in the USA. Roots were planted in jars containing 10 cm of soil and grown under fluorescent ballast lamps (DRLF-400) suitable for photosynthesis (12 hour day). The temperature was maintained at 20-22° C at night and 25-27° C during the day.

#### *Virulence screening.*

Virulence screening was conducted in three stages.

1. In the first stage, short (1-2 cm) sections of *E. esula* stems with several leaves were surface sterilized and placed in a petri dish containing sterile medium. The tissue was inoculated with an aqueous suspension of fungal spores. Control tissue was inocu-

lated with sterile medium. The inoculated tissue was incubated for 10 days and scored for disease development on a scale of 0-3, where 0 = absence of any damage, 1 = few small lesions or discoloration of plant tissue, 2 = >50% of the tissue sample was infected with fungal mycelium and 3 = the entire tissue sample was colonized by the fungal mycelia. "Symptoms" on the plant samples included chlorosis and necrosis. The most virulent isolates (51 of 565, ca 10%) progressed to stage two screening.

2. At the second stage, whole stems of *E. esula* (15-20 cm long with more than 10 leaves) were placed in sterile test tubes containing liquid cultures of the selected fungi. Control plants were placed in sterile medium. The plants were incubated for 10 days and the results were recorded using the disease index. The most virulent strains (6 of 51) progressed to stage three screening.
3. At the third stage, whole potted plants were inoculated. Colonized toothpicks were prepared by placing a sterile toothpick in the center of a PDA culture of each of the selected pathogens. After several days incubation, the toothpicks were covered with fungal mycelia. The toothpicks were collected and dried. Plants were inoculated by attaching a colonized toothpick to the stem of the potted plant. The plant was damaged at the attachment site by cutting with a sterile scalpel. The plants were covered with a plastic cylinder for the first 24 h after inoculation. Inoculated plants were evaluated periodically for 30 days.

## Results

A total of 565 fungal strains were tested for virulence to *E. esula* in the first stage of the virulence screening. Fifty-one of these strains colonized and destroyed excised tissue of *E. esula*. Control samples of the excised *E. esula* did not show symptoms of chlorosis or necrosis for at least one month. These 51 isolates progressed to the second stage of the virulence screening.

In the second stage virulence study, the average disease index of six of the isolates was greater than 2.5 on the scale of 0-3. None of the tested strains was virulent enough to destroy 100% of the *E. esula* stems. All six of these virulent isolates were isolated from diseased plant samples collected in Krasnodar territory, close to the presumed center of origin of *E. esula* (Gassmann and Schroeder, 1995).

Finally, only three of the 6 strains tested showed a mean disease index of greater than 2 on a scale of 0-3 in the third stage of virulence screening.

These three isolates killed or seriously damaged inoculated plants under laboratory conditions and will be further investigated for control of *E. esula* in biocontrol studies in Russia and in the US.

The efficacy of this virulence screen was tested. The virulence of nine fungal strains rejected in the preliminary stages was evaluated against intact *E. esula* plants (stage 3). The mean virulence score of these rejected strains was 0.56 and did not significantly differ from the average of the controls (0.36). This result suggest that the strains rejected at preliminary stages of the screening do not have potential for biocontrol and thus the method of three-stage express screening does not lead to loss of promising strains.

## Discussion

Two conclusions can be made from our study.

1. Three-stage express screening of virulence saved time and labor, as well as mini-

mized the number of plants necessary to select pathogens from a large pool of fungal isolates.

2. This three-stage screening produced promising results. Three strains were selected that are virulent to *E. esula* and will be further evaluated as potential biocontrol agents for control of leafy spurge in Russia and the US.

### References

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