WEEDS AS AN INOCULUM SOURCE OF *SCLEROTINIA SCLEROTIORUM*

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ABSTRACT

Numerous weed species could be alternative hosts for diseases of cultivated plants, among which fungi play an important role (Anikster 1982, Jenkinson and Parry 1994, Roy *et al.,* 1994, 1997). Velvetleaf (*Abutilon theophrasti* Medik.), ragweed *(Ambrosia artemisiifolia* L.) and rough cocklebur (*Xanthium strumarium* L.) are very aggressive weeds in arable crops such as soybean, sunflower, maize and sugar beet.
Velvetleaf, ragweed and rough cocklebur plants infected with *Sclerotinia sclerotiorum* (Lib.) de Bary (Sclerotinia stem rot, white mold) were recorded on several locations in eastern Croatia during 2001 and 2002. Symptoms on velvetleaf plants occured on basal stem parts as well as on upper plant parts, fruits and seeds. Symptoms of white mold on ragweed and rough cocklebur plants occured only on stems. Isolates of *S. sclerotiorum* from diseased weed plants were used as inoculum sources for pathogenicity tests on soybean and sunflower. Pathogenicity tests were done in laboratory and field conditions. Inoculated plants were examined daily to record development of lesions, wilting and lodging. On sunflower plants first lesions in field and laboratory were recorded on the second day after inoculation with isolates from velvetleaf and ragweed and on the third day after inoculation with isolate from rough cocklebur. On soybean plants first symptoms in laboratory were recorded on the second day and in the field on the fourth day after inoculation with isolates from velvetleaf and ragweed. First lesions on soybean after field inoculation with isolate from rough cocklebur was recorded on the third day. Total number of lodging plants showed that all examined isolates were more pathogenic on sunflower than on soybean.
Occurence of white mold in velvetleaf, ragweed and rough cocklebur can increase inoculum density of *S. sclerotiorum* in soil.

Key words: isolates pathogenicity, *S. sclerotiorum*, weeds

IZVLEČEK

PLEVELI KOT VIR OKUŽBE Z BELO GNILOBO (*SCLEROTINIA SCLEROTIORUM*)

Številne vrste plevelov so lahko sekundarni gostitelji bolezenskim povzročiteljem kmetijskih rastlin, med katerimi imajo pomembno vlogo glive (Anikster 1982, Jenkinson and Parry 1994, Roy *et al*., 1994, 1997). Baržunasti oslez (*Abutilon theophrasti* Medik.), navadna ambrozija *(Ambrosia artemisiifolia* L.) in navadni bodič (*Xanthium strumarium* L.) so zelo trdovratni pleveli, na primer v posevkih soje, sončnic, koruze in sladkorne pese.
V letih 2001 in 2002 smo na nekaj lokacijah v vzhodnem predelu Hrvaške našli baržunasti oslez, navadno ambrozijo in navadni bodič, ki so bili okuženi z belo gnilobo (*Sclerotinia sclerotiorum* [Lib.] de Bary). Simptomi okužbe na rastlinah baržunastega osleza so se razvili tako na bazalnem delu stebel kakor tudi na zgornjih delih rastlin, na plodovih in semenih. Na rastlinah navadne ambrozije in navadnega bodiča so se simptomi okužbe z belo gnilobo razvili le na steblih. Izolati glive *Sclerotinia sclerotiorum* iz okuženih plevelov so služili kot vir okužbe za teste patogenosti na soji in sončnicah, v laboratorijskih in poljskih poskusih. Na okuženih rastlinah smo dnevno beležili pege, venenje in poleganje. Na sončnicah so bili prvi simptomi na polju in v laboratoriju vidne drugi dan po okužbi z izolati glive iz baržunastega osleza in navadne ambrozije in tretji dan po okužbi z izolatom iz navadnega bodiča. Na soji so se simptomi v laboratoriju pokazali drugi dan, na polju pa četrti dan po okužbi z izolati iz baržunastega osleza in navadne ambrozije. Prve poškodbe na soji po okužbi na polju z izolatom iz navadnega bodiča so bile vidne tretji dan. Skupno število poleglih

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rastlin je pokazalo, da so bili vsi testirani izolati bolj patogeni za sončnico kakor za sojo. Okuženost baržunastega osleza, navadne ambrozije in navadnega bodiča z belo gnilobo lahko poveča infekcijski potencial glive v tleh.

Ključne besede: patogenost izolatov, *S. sclerotiorum*, pleveli

1 INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is a parasite which presence was recorded on a total 408 different plant species of 278 genus and 75 families (Bolland and Hall, 1994). A large number of cultivated plants are hosts to *S. sclerotiorum*, such as sunflower, soybean, oilseed rape, tobacco plant, tomato, salad, cucumbers, common lentil, common bean, alfalfa, tulips, lilies, etc. It is also important to point out that *S. sclerotiorum* is hosted by weeds, some of which are widespread and very aggressive in our country. In eastern Croatia the occurrence of *S. sclerotiorum* was recorded on weeds *Abutilon theophrasti* Medick., *Ambrosia artemisiifolia* L. and *Xanthium strumarium* L. (Jurković and Culek, 1997, Jurković *et al.,* unpublished). Occurrence of the named parasite may be of considerable importance; on the one hand, for its widespread, and on the other hand, for herbicides that are in many cases insufficiently efficient.

Literature published on this matter provides information that weeds could not only provide alternative hosts for many parasites to cultivated plants, but also they play an important role in disease epidemiology as a source of inoculum and as an epidemiological bridge between two vegetations (Dinoor, 1974).

The aim of our research was to determine weeds that provide a host to *S. sclerotiorum* and to compare pathogenicity of isolates harboured by the weeds (*A. theophrasti* and *A. artemisiifolia*) to sunflower and soybean with isolates from sunflower and soybean.

2 MATERIALS AND METHODS

Plants of *A. theophrasti* and *A. artemisiifolia*, which showed disease symptoms, were collected in 2001 and 2002 from three sites in eastern Croatia. Infected plant tissues and sclerotia were washed under running tap water for 30 minutes, than surface sterilized in 70% ethanol for 30 seconds, rinsed in distilled water and left to dry at room temperature. Parts of plant tissues and sclerotia were placed in Petri dishes on PDA (pH 6.2-6.5). They were incubated in thermostat at 25°C under a 12 hour light/dark regime.

In order to inoculate soybean plants, a two day old culture of *S. sclerotiorum* was used. The trial was set according to Kim *et al.,* (2000). Twenty soybean plants were inoculated with *S. sclerotiorum* isolate from *A. artemisiifolia* (A), further 20 soybean plants were inoculated with *S. sclerotiorum* isolate from *A. theophrasti* (Ab), and the last 20 soybean plants were inoculated with *S. sclerotiorum* isolate from soybean (S). The plants were infected at growth stage V1 (Fehr *et al*., 1977). Small agar plugs with mycelium were used to infect healthy plants, so that those agar plugs were pressed on the cotyledon of each plant. Inoculation site was covered with cotton wool moistured with distilled water, a piece of aluminium foil and a PVC bag for 44 hours. Control plants were also wrapped with cotton wool, foil and a bag, but pure agar plugs were used.

Artificial infection of sunflower plants was done according to Jurković and Culek (1997). For sunflower inoculation four day old *S. sclerotiorum* cultures were used. The size of agar plugs with mycelium was 5x5 mm. Inoculation of sunflower stems was done on three week old plants. The same number of plants (20) was inoculated with isolates of *S. sclerotiorum* from sunlower (Su), *A. theophrasti* (Ab) and *A. artemisiifolia* (A). Inoculation site was covered with cotton wool moistured with distilled water, a piece of aluminium foil and a PVC bag for 4 days. Instead of agar plugs with a *S. sclerotiorum* isolate, pure PDA plugs were used for control plants.

All trials were done both in the laboratory and in the field.

All infected plants were inspected on a daily basis: soybean plants in a seven day period, and sunflower plants in a nine day period. Plants were also examined daily to record wilting, while the size of lesion was measured on soybean on 2^{nd}, 5^{th} and 7^{th} day, and on sunflower on 3^{rd}, 5^{th}, and 9^{th} day.

3 RESULTS AND DISCUSSION

With regard to natural infection conditions the first symptoms of disease in *A. theophrasti* were observed at the end of June: they appeared between 2nd and 3rd internodium in form of brown-grey spots. Their size varied from 1-2 to 10-20 cm. Their ringlike development on the infected stem led to disintegration of tissue. Leaves of diseased plants lost their turgor, hanging on the stems and wilted in the course of time. The symptoms observed on stalks were identical to those recorded on the stem, and in the final phase of disease sclerotia developed. In later growth stages infected plants could be easily detected, because their stem parts were bleached almost in its full length, their pith was destroyed and numerous sclerotia developed both in it and on stem surface. Stems broke easily to the touch and sclerotia fell off.

The first symptoms of disease in *A. artemisiifolia* were observed at the beginning of July and were identical to those found on *A. theophrasti*. Only the stem was infected on *A. artemisiifolia*, while the symptoms of infection on *A. theophrasti* were also established on basal stem parts and in seeds. The first symptoms of disease on soybean were observed two days after inoculation in the laboratory, and on 4th day in the field. Infected plants were characterized by watery brown spots that spread on the stem hemispherically, which led to wilting and lodging. Dynamics of lodging of soybean plants both in the field and laboratory is shown in Figure 1. *S. sclerotiorum* isolate isolated from soybean (S) proved to be the most pathogenic to soybean. In the field there were 14 lodged plants after a seven day period (S) and with A and Ab isolates there were 9 respectively 10 lodged plants. Control plants were characterized by no changes at all. Mean value of lesion size on soybean plants is shown in Figure 2. On the second day after inoculation the lesion size on all inocuated plants were about equal (0.4-0.7 cm). On 7th day of inoculation the difference in lesion size was more than obvious, depending on the isolate itself. Mean lesion size on plants inoculated with isolates A and Ab ranged from 1.9 and 2.2 cm. On the other hand, lesions on plants inoculated with isolate S were significantly bigger, accounted for 3.1 cm.

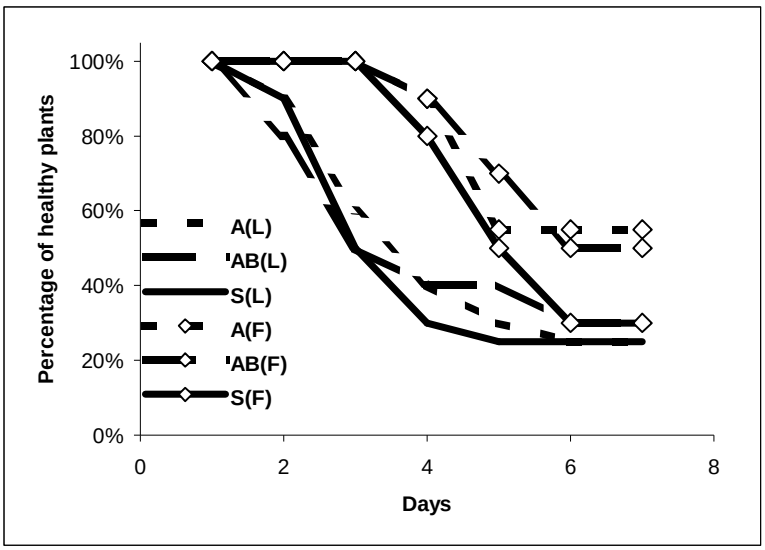

Figure 1: Dynamics of lodging of soybean plants both in the field and laboratory
Legend: A(L) - isolate of *A. artemisiifolia* (laboratory results), AB(L) - isolate of *A. theophrasti* (laboratory results), S(L) - isolate of soybean (laboratory results), A(F) - isolate of *A. artemisiifolia* (field results), AB(L) - isolate of *A. theophrasti* (field results), S(L) - isolate of soybean (field results)

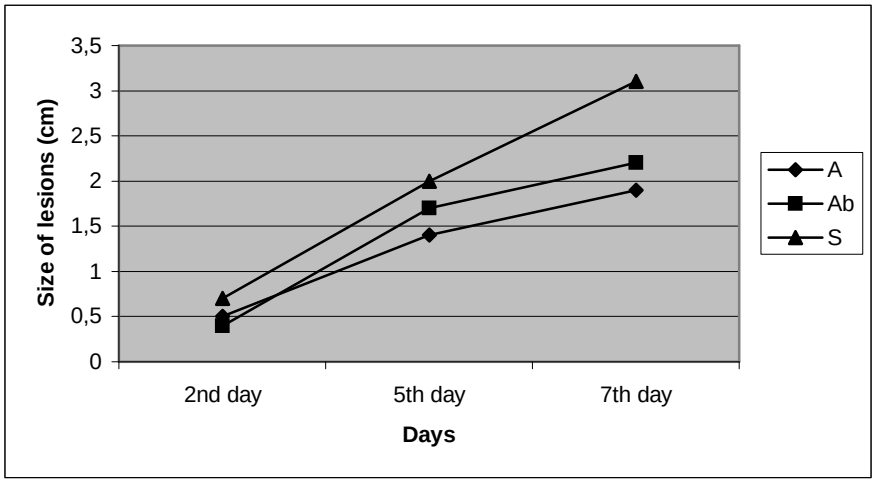

Figure 2: Mean value of lesion size on soybean after inoculation with isolates of *S. sclerotiorum* in the field
Legend: A- isolate of *Ambrosia artemisiifolia*, Ab- isolate of *Abutilon theophrasti*, S- isolate from soybean

The first symptoms of disease on sunflower both in the field and laboratory were observed also in the form of watery spots on the second day of inoculation. Wilting of plants was recorded on 3rd day in the laboratory, and on 4th day in the field. Dynamics of lodging of sunflower plants both in the field and laboratory is shown in Figure 3.

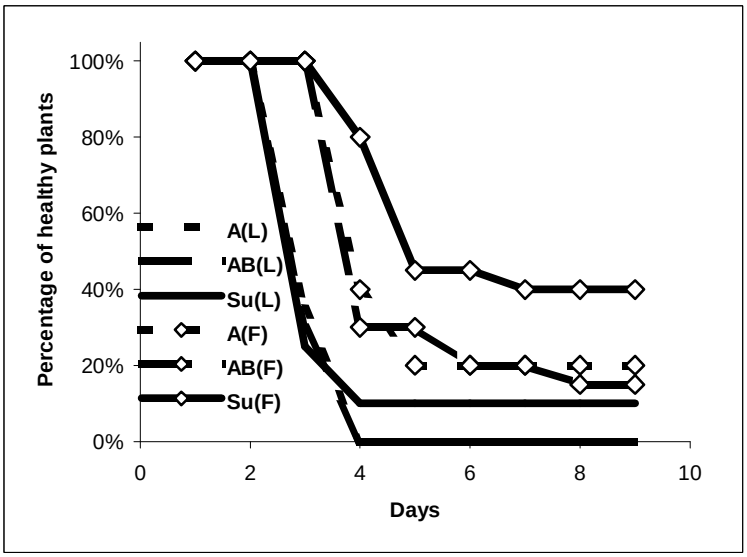

Figure 3: Dynamics of lodging of sunflower plants both in the field and laboratory
Legend: A(L) - isolate of *A. artemisiifolia* (laboratory results), AB(L) - isolate of *A. theophrasti* (laboratory results), Su(L) - isolate of sunflower (laboratory results), A(F) - isolate of *A. artemisiifolia* (field results), AB(L) - isolate of *A. theophrasti* (field results), Su(L) - isolate of sunflower (field results)

Sunflower plants that broke as a result of parasitic infection were characterized by disintegrated tissue and mycelium of the parasite was developed abundantly.
Mean value of lesion size on sunflower plants is shown in Figure 4. On 3rd day mean value of lesion size on the infected sunflower plants varied from 1.8 cm (isolate Su) to 2.7 cm

(isolate Ab). On 9th day the highest mean value of lesion size was measured on plants inoculated with isolate Ab (10.1 cm), lesions on the plants inoculated with isolate A were somewhat smaller (9.4 cm), whereas the smallest lesions were measured on the plants inoculated with isolate Su (6.9 cm).

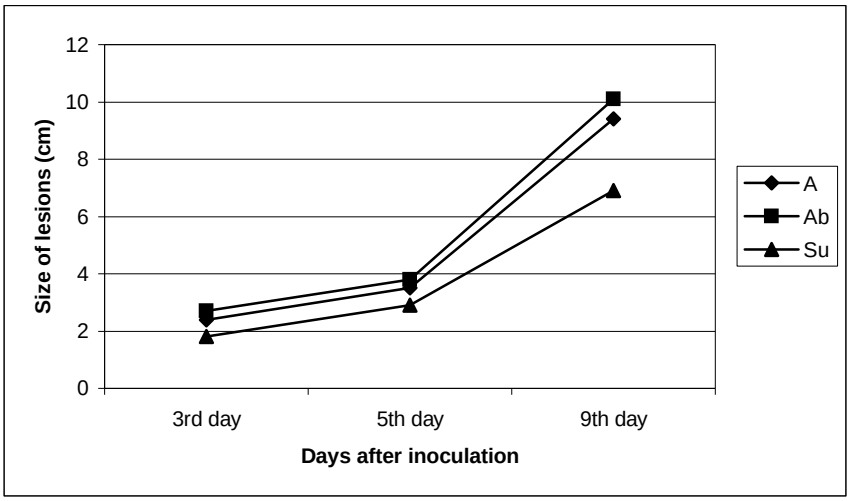

Figure 4: Mean value of lesion size on sunflower after inoculation with isolates of *S. sclerotiorum* in the field

Legend: A- isolate of *Ambrosia artemisiifolia*, Ab- isolate of *Abutilon theophrasti*, Su- isolate from sunflower

Isolates from weeds *A. theophrasti* and *A. artemisiifolia* were stronger pathogenic to sunflower than isolates from sunflower, which corresponds to Jurković and Culek (1997). Results of our research proved that *A. theophrasti* and *A. artemisiifolia* are alternative hosts for *S. sclerotiorum*. In conditions of natural infection, there are symptoms on diseased weeds that are similar to those that appear on diseased soybean and sunflower plants: rot of the basal stem part and "white rot". On the other hand, when it comes to artificial infection of soybean and sunflower both in the field and in laboratory, it is established that disease may be reproduced with isolates from weeds (A and Ab isolates), and the symptoms are similar to those of natural infections. There were some other investigations done, according to which isolates of the pathogens from infected weeds could be even more pathogenic to cultivated plants than isolates from those plants themselves (Hepperly *et al.*, 1980, Sackston and Wylmore 1990, Ćosić 2001).

4 SUMMARY

Weeds *A. theophrasti* and *A. artemisiifolia* are alternative hosts to *S. sclerotiorum*. By artificial infection of soybean and sunflower plants in the field and laboratory it is established that disease may be reproduced on cultivated plants with isolates from weeds, and the symptoms are identical to those which appear after natural infection. Results of our investigation show that *S. sclerotiorum* isolates from *A. theophrasti* and *A. artemisiifolia* could be even more pathogenic to cultivated plants than isolates from those plants themselves.

5 REFERENCES

Anikster, Y. 1982. Alternate hosts of *Puccinia hordei*. Phytopathology 72: 733-735.

Boland, G. J., Hall, R. 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. Canadian Journal of Plant Pathology, 16: 93-108.

Ćosić, J. 2001. Taksonomija *Fusarium* vrsta izoliranih s kultiviranog bilja, korova i njihova patogenost za pšenicu. Doktorska disertacija, Poljoprivredni fakultet u Osijeku.

Dinoor, A. 1974. Role of Wild and Cultivated Plants in the Epidemiology of Plant Diseases in Israel. Annual Review of Phytopathology:413-436.

Fehr, W. R., Caviness, C.E. 1977 Stages of soybean development. Iowa Agric. and Home Econ. Exp. Stn. Spec. Rep. 80.

Hepperly, P. R., Kirkpatrick, B. L., Sinclair J. B. 1980. *Abutilon theophrasti*: Wild Host For Three Fungal Parasites of Soybean. Phytopathology 70: 307-310.

Jenkinson, P., Parry, D. W. 1994. Isolation of *Fusarium* species from common broad-leaved weeds and their pathogenicity to winter wheat. Mycol. Res. 98 (7): 776-780.

Jurković, D., Culek, M. 1997. *Abutilon theophrasti* Medik.- a new host for *Sclerotinia sclerotiorum* (Lib.) de Bary in Croatia. Acta Phytopathologica et Entomologica Hungarica, 32: 307-312.

Kim, H. S., Hartman, G. L., Manandhar, J. B., Graef, G. L., Steadman, J. R., Diers, B. W. 2000. Reaction of Soybean Cultivars to *Sclerotinia* Stem Rot in Field, Greenhouse, and Laboratory Evaluations. Crop. Sci. 40: 665-669.

Krupinsky, J. M. 1987. Pathogenicity on wheat of *Pyrenophora tritici-repentis* isolated from *Bromus inermis*. Phytopathology 77: 760-765.

Sackston, W. E., Wylmore, L. A. 1990. Velvetleaf (*Abutilon theophrasti*) potential inoculum reservoar of *Verticillium* spp. pathogenic to crops. Phytopathology, 80: 123.