PRELIMINARY RESULTS REGARDING VIRULENCE TEST OF SOME *VERTICILLIUM LONGISPORUM* STAINS ON *BRASSICA NAPUS*

REZULTATE PRELIMINARE PRIVIND TESTAREA VIRULENTEI UNOR TULPINI DE *VERTICILLIUM LONGISPORUM* LA *BRASSICA NAPUS*

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Abstract. *Verticillim longisporum is soil-borne vascular fungal pathogens with host specify to cruciferous plans such oilseed rape (Eynck et al.2008). The fungus is able to survive in soil for several years through the production of microsclerotia and because there are no approved fungicides for these diseases, it is important to cultivate some resistant cultivars. The aim of this study was to test the virulence of 3 Verticillium longisporum isolates, in order to identify which is the most aggressive isolate for Brassica napus. For this purpose, we used two oilseed rape cultivars one which is less susceptible to the disease (Express) and one, which is highly susceptible to the disease (Falcon). The plants were artificial infected which each V.longisporum isolate by root deep inoculation under controlled conditions. For four weeks, the plants were scored for the symptoms using an assessment key with nine classes. Based on the obtained results the most aggressive stain proved to be VL43.*
Key words: virulence, test, *Verticillium longisporum*

Rezumat. *Verticillium longisporum este un agent patogen vascular care se găseşte în sol şi are plante gazda plante din familia Crucifere cum ar fi rapita (Eynck et al.2008). Prin intermediul microscleroţilor, ciuperca poate supravieţui în sol mai mulţi ani, iar datorită falptului că înca nu exsită un control chimic al bolii, este importantă cultivarea de soiuri cu rezistenţă la această boală. Scopul acestui studiu a fost testarea a trei tulpini de Verticillium longisporum pentru a identifica care este cea mai virulentă la specia Brassica napus. Pentru realizarea acestui lucru, s-au utilizat cultivarele de rapiţă Express cu rezistenţă moderată la această boală şi Falcon care este foarte sesibil la această boală. Plantele au fost inoculate artificial cu cele trei tulpini de Verticillium longisporum prin metoda tăierii rădăcinilor in condiţii controlate. Timp de patru săptamâni, plantele au fost bonitate folosind o clasă de bonitare cu nouă note. În urma observatiilor facute cea mai agresiva tulpina s-a dovedit a fi VL43.*
Cuvinte cheie: virulenta; testare; *Verticillium longisporum*

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INTRODUCTION

Verticillium wilt caused by the fungal pathogen *Verticillium longisporum* is one of the most important pathogens of oilseed rape (*Brassica napus*) from northern Europe (Rygulla et al., 2007). The funguses enter in the vascular system of the plant and moves into the xylem vessels causing wilting symptoms, and in the later stages premature ripening of the seed. In this paper, we tested three isolates of *Verticillium longisporum* in order to identiy the most aggressive stain for oilseed rape cultivars.

MATERIAL AND METHODS

The artificial infection was performed with three isolates of *Verticillium longisporum*, two of them (VL 9802 and VL 4329) provided by BCCM/IHEM Scientific Institute of Public Health Section Mycology and Aerobiology, Brusseles and one (VL43) from The Division of Plant Pathology and Plant Protection, Georg August University, Gottingen, Germany.

The inoculum for each isolate was performed using 250 ml PDB (potato dextrose broth) medium, which was inoculated with 500 µl spore stock solution. The cultures were incubated 7 days at 23^0C on a rotary shaker. After 7 days, the culture was filtered and using a haematocytometer, we determined the spore concentration. For the inoculation, we diluted the solution at $1x10^6$ spore mL^{-1}.

As plant material we used two *Brassica napus* cultivars Express which is less susceptible to *V. longisporum* and Falcon which is highly susceptible. The seeds from each cultivar were surface sterilized by immersion in ethanol 70% for 2 minutes and then rinsed two times in distillated water.

The seed were sown in silica sand and grown in the climatic chamber at 23^0C. 10 days old plantlets were carefully washed from sand. The inoculation was made by cutting 2 cm from the root apex and holding them for 30 min in *Verticillium longisporum* spore suspension. We used for each isolate 10 inoculated plants and 10 control plants. At the control plants, the root apex was also cut and the plans were hold 30 min in distillated water.

After inoculation the plants were transferred into pots containing a mixture of compost, peat and sand (2:1:1) and grown in a climatic chamber at 23^0C with a light/dark cycle of 14/10. Every week we take the disease scores using an assessment key with nine classes as described by Eynck et al 2007 (table 1).

Table 1

Assessment key for scoring disease severity

Score	Symptom development
1	The plant has no symptoms
2	The oldest leaves have slight symptoms (yellowing, black veins)
3	The next younger leaves have slight symptoms
4	About 50% of the leaves have symptoms
5	More than 50% of the leaves have symptoms
6	Up to 50%of the leaves are dead
7	More than 50% of the leaves are dead
8	Only apical meristem is still alive
9	The plant is dead

RESULTS AND DISCUSSIONS

Based on the disease scores that were taked every week we calculated the area under disease curve (AUDPC) in order to determnine which is the most aggresive isolate of *Verticillium longisporum* for the species *Brassica napus*.

In case of the isolate VL9802 no typical symptoms were observed during the four weeks of the scoring. In the last week, the disease score of the infected plants were „1" – no symptoms and we observed no difference between the infected plants and the control plants.

The calculated AUDPC values from the disease scores were 0 in all the infected variants (fig.1).

In the case of the isolate VL4329, typical symptoms like asymmetric yellowing of leaves and early stunting started to be observed at 21 days after infection. The values the calculated AUDPC were higher than at the isolate VL9802 (fig.1). For this isolate the susceptible cultivar Falcon had disease scores between 3-6 after 28 dpi (days after infection) and Express had values between 1 and 3 at 28 dpi.

The isolate VL 43 had the higher values of the calculated AUDPC. In this case, typical symptoms started to appear at 14 days after infection. The disease scores and the calculated AUDPC values were the most higher for the both oilseed rape cultivars.

Regarding the evolution of the plant height for all the isolates, the results are presented in fig. 2.

The biggest values for the plant height were in the case of the isolate VL9802 and the smaller values were in the case of the isolate VL43. As it is known, one of the typical symptoms of the infection with *Verticillium longisporum* is the early stunting and as we can observe the evolution of the plant height in the case of the isolate VL43, we can affirm that this isolate was the most aggressive.

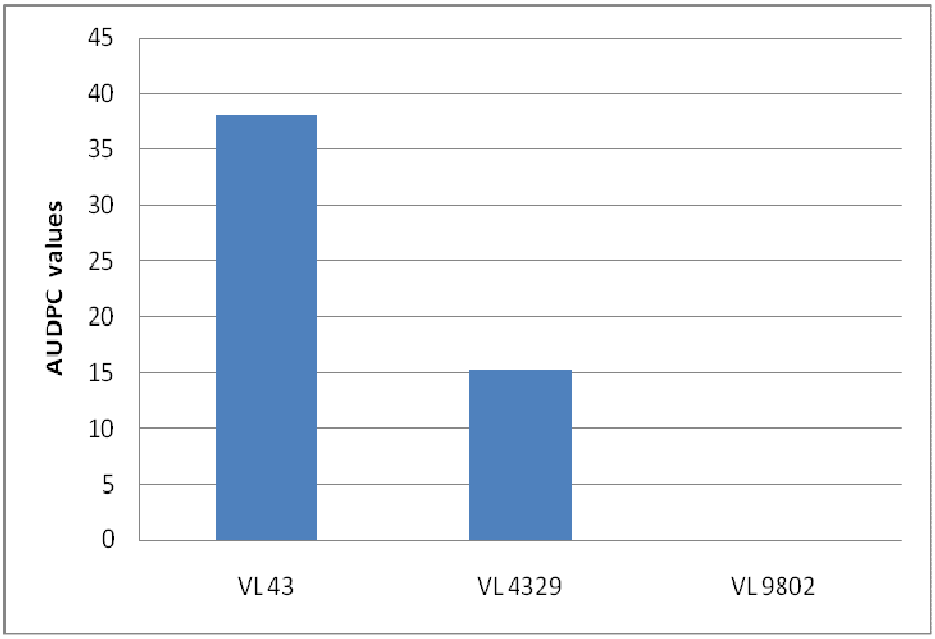

Fig. 1 – The evolution of the virulence of the three isolates of *Verticillium longisporum*

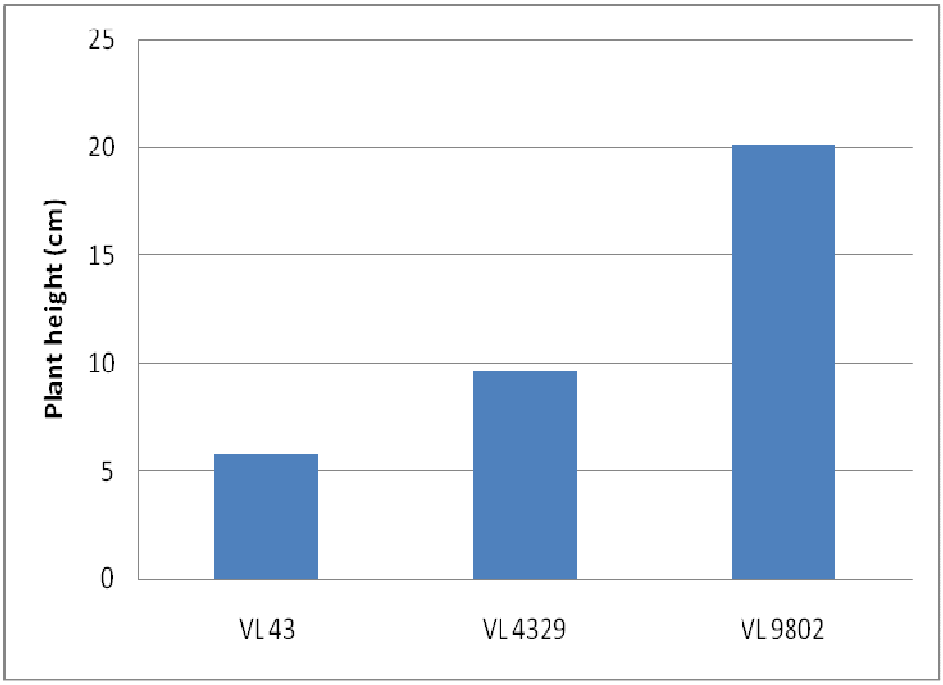

Fig. 2 – Evolution of the plant height of the inoculated plants with the three isolates of *Verticillium longisporum*

CONCLUSIONS

In this study we tested three isolates of *Verticillium longisporum* on two *Brassica napus* cultivars in order to identify the most aggresive isolate for this species. Our virulence test performed in the climatic chamber demonstrated that the isolate VL43 originating from Gottingen is the most aggresive producing the most major losses. At this isolate, it could be also observed that the most typical symptoms of the disease like yellow leaves, chlorosis, black veins on the back of the leaves and early stunting started to appear at 14 dpi. Eynck (2007) also reported this and Zhou (2006) in their studies carried out with isolates of both *V. longisporum* and *V. dahlia* on plants. Symptoms of chlorosis and vein blackening were evident after 14 days and stunting was apparent 21 days after inoculation with V. *longisporum*.

In case of the isolate VL9802 that produced no symptoms we can affirm that this isolate is not aggressive for oilseed rape but is may be aggressive for other plants from *Brassicacace* family. In conclusion, the isolate that we will use for our further studies is VL43 from Gottingen.

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REFERENCES

1. Burlacu (Arsene) M. C., Leonte C., Morariu A., Calistru A., Simioniuc D., 2010 - *Researches regarding the analysis of the factors that influence embryogenesis in microspore cultures of Brassica napus.* Lucrari Stiintifice U.S.A.M.V. Iasi , vol 53, nr. 1, p. 36-39.

2. Burlacu (Arsene) M. C., Calistru A., Leonte C., 2011 - *Evaluation of the genetic diversity among some oliseed rape Brassica napus cultivars revealed by RAPD markers compared with morphological traits evaluation.,* Lucrari Stiintifice U.S.A.M.V. Iasi, vol 54, nr 1.

3. Debode J., Declercq B., Höfte M., 2005 - *Identification of cauliflower cultivars that differ in susceptibility to Verticillium longisporum using different inoculation methods***. J.** Phytopathol. 153, 257-263.

4. Eynck C., Koopmann B., Grunewadt-Stöcker G., Karlovsky P., Tiedemann A, 2007- *Differential interactions of Verticillium longisporum and Verticillium dahliae with Brassica napus detected with molecular and histological techniques***.** Eur. J. Plant Pathol. 118, p. 259-274.

5. Eynck C., Koopman B., Tiedemann A., 2008 – *Identification of Brassica accessions with enhanced resistance to Verticillium longisporum under controlled and field conditions*. Journal of Plant Diseases and Protection, 116(2), p. 63-72.

6. Leonte C., Burlacu (Arsene) M., Simioniuc D., Vatavu R., Calistru A., 2010 - *Evaluation of the genetic similarity of some Brassica naspus cultivars using RAPD markers,* Enviromental Engineering and Management Journal, 9.

10. Rygulla W., Showdown R., Eynick C., Koopmann B., Tiedemann A., Luhs W. And Friedt W., 2007 – *Broadening the genetic basis of Verticillium longisporum resistance in Brassica napus by interspecific hibridization***.** Plant Breeding 126, p. 596-602.

11. **Steventon L.A., Happstadius I., Okori P., Dixelius C., 2002** - *Development of a rapid technique for the evaluation of the response of Brassica napus to Verticillium wilt.* Plant Disease. 86, p. 854-858.
12. **Subbarao K.V., Chassot A., Gordon T.R, Hubbard J.C., Bonello P., Mullin R., *Okamoto D., Davis R.M, Koike S.T. , 1995*** - *Genetic relationships and cross pathogenicities of Verticillium dahliae isolates from cauliflower and other crops.* Phytopathology 85, p. 1105-1112.
13. **Zhou L., Hu Q., Johannson A., Dixelius C., 2006** - *Verticillium longisporum* and *Verticillium dahliae*: infection and disease in *Brassica napus*. *Plant Pathology* 55, p. 137-144.