Short Communication

Determination of the Host Range of *Fusarium moniliforme* Isolated From Winter Wild Oat (*Avena ludoviciana*) in Iran

H. Kazemi and P. Shimi

Plant Pests and Diseases Research Institute, Tehran, Iran

(Received 2 November 2004; returned 16 January; accepted 9 March 2005)

An isolate of *Fusarium moniliforme*, a pathogen of winter wild oat (*Avena ludoviciana*), was obtained from Tehran Province, Iran, in 1994. A host range test performed on wheat, barley, maize, rye, millet, crested wheatgrass, faba bean, red bean, green bean, sunflower, soybean, oilseed rape, cotton, safflower, cucumber, water melon, berseem clover, and sainfoin, resulted in no symptom induction by the pathogen. However, winter wild oat, crested wheatgrass, johnsongrass and tomato showed susceptibility to the pathogen with 78, 24, 19 and 17% mortality, respectively. The results indicate that this pathogen could be considered as a potential biological agent for the control of winter wild oat.

Key words: winter wild oat, *Avena ludoviciana*, *Fusarium moniliforme*, biocontrol.

در سال ۱۳۷۵، از نمونه‌های یولاف وحشی زمستانه (*Avena ludoviciana*) در استان تهران که دارای علائم بیماری بودند، ایزوله‌هایی از قارچ *Fusarium* spp. جداسازی و تعیین نام شد. در بین آنها، گونه *Fusarium moniliforme* دارای بیشترین فراوانی بود. آزمایش‌های بیماری‌زایی بر اساس اصول کخ نشان داد که این قارچ پاتوژن یولاف وحشی می‌باشد. در تعیین دامنه میزبانی، مایه‌زنی این قارچ به ارقامی از گندم، جو، ذرت، چاودار، ارزن علوفه‌ای، باقلا، لوبیا قرمز، لوبیا سبز، آفتابگردان، سویا، کلزا، پنبه، گلرنگ، خیار، هندوانه، شبدر برسیم و اسپرس، منجر به بروز علائمی در آنها نشد. ولی، این پاتوژن در یولاف وحشی، قیاق، بیدگیاه و گوجه فرنگی به ترتیب موجب ۷۸، ۱۹، ۲۴ و ۱۷ درصد مرگ گیاهچه‌ها شد. این نتایج نشان داد که ممکن است بتوان از ایزوله یاد شده به عنوان عامل بیوکنترل یولاف وحشی استفاده نمود.

Correspondent to: P. Shimi, E-mail: Shimi@ppdri.ac.ir

Many microorganisms (bacteria and fungi) are pathogens of weeds and/or produce phytotoxic compounds that have a potential application in the biological control of weeds (Hoagland, 1990). Many fungal species, especially *Fusarium* spp. are common and widespread in plants, plant products, and soils throughout the world. Grain crops (Abbas & Mirocha, 1985; Vesonder, 1986; Abbas *et al*., 1989) and weeds (McCain, 1978; Jones & Hancock, 1990) are frequently infected pathogenically or colonized saprophytically by *Fusarium* spp.

Some *Fusarium* species such as *F. lateritium*, *F. oxysporum* and *F. solani* isolated from infected plant parts and soil, play an important role in the biological control of weeds such as velvetleaf (*Abutilon theophrasti* Medicus), prickly sida (*Sida spinosa* L.),Texas gourd [*Cucurbita texana* (Scheele) Gray], and jimsonweed (*Datura stramonium* L.) (Boyette *et al.*, 1984; Boyette & Walker, 1985; Jones & Hancock, 1990; Abbas *et al.*, 1991).

Members of the genus *Fusarium* produce a range of phytotoxic compounds that are chemically diverse and possess a broad range of activities and metabolic effects. For example, moniliformin causes growth inhibition, necrosis, and chlorosis on many plants (Duke, 1986; Hoagland, 1990). Fusaric acid isolated from *Fusarium*-infected plants is a well-known phytotoxin and causes wilt in several plants (Kuo & Scheffer, 1964, Davis, 1969, Chakrabari & Basuchaudharg, 1980).

Winter wild oat (*Avena ludoviciana* Dur.) is an economically important weed, especially in the wheat fields of Iran where it can reduce yields by over 30% (Zand *et al*., 2002). Winter wild oat is mainly controlled by using chemical herbicides which can have side effects on ecosystems. Therefore, biocontrol of this weed would be amore environmentally friendly approach. This project set out to discover locally active pathogens on winter wild oat for use as a potential biocontrol agent.

From the fall of 1994 to the spring of 1995, farms in the Tehran province were surveyed and winter wild oat collected. The plant samples were transferred to the

laboratory for the following experiments: 1) Samples were washed under running tap water for 15 minutes, then 5-mm pieces of each sample were surface sterilized with a 2 % sodium hypochlorite solution for 3 minutes. The pieces were washed three times with sterile distilled water and dried on sterile filter paper. The samples were then cultured on Potato Dextrose Agar (PDA), Corn Meal Agar (CMA) and Nash & Snyder culture media (Nelson *et al*., 1983) and incubated for 24-48 hours at 24 °C. 2) Pieces of plant tissue at the border of infected areas were cut out and placed on Tap Water Agar (TWA) and Nash & Snyder culture media and incubated for 24-48 hours at 24 °C. Samples of developing fungi were transferred aseptically to PDA and then purified using microscopic single-spore isolation.

Isolated fungi were identified using "*Fusarium* species, an illustrated manual (Nelson *et al.*, 1983)". Spore production for pathogenicity tests on host plants was carried out as follows: PDA cultures (7-10 days old) were homogenized at 22-24 °C in 50 ml of distilled water. The suspension included conidia and mycelia in which the concentration was adjusted to 1.5×10^6 conidia ml^{-1} of distilled water. A second inoculum was prepared by growing the isolate on autoclaved wheat and barley grain. Equal amounts of wheat and barley grain were mixed and soaked in water for 15 hours. Excess water was removed and the grain was autoclaved for 50 minutes at 121 ± 1°C in flasks (approximately 100g mixture L^{-1}). Pieces of agar culture were added to the grain and incubated for 14 days at 24 °C. After 3 days, the flasks were shaken daily.

Forty 20-cm diameter pots were filled with pasteurized soil. Six seeds of wild oat were planted in each pot. Fungal inoculation was carried out as described by Dhingra and Sinclair (1994): a) Infestation of pasteurized soil (2 grams of inoculum per 100 grams of soil) before sowing. b) Direct inoculation of the plant crown at the 2-4 leaf stage with 2 ml of inoculum suspension using a disposable syringe and 22-gauge needle. c) Dipping the root system of the plant at the 2-4 leaf stage in an inoculum suspension for 1 minute. The control pots were treated with sterile distilled water. To create high humidity (85% RH) in all experiments, the

pots were kept under plastic covers after inoculation. The pots were kept in a glasshouse at 25/32 °C (night/day).

The developing pathogen was re-isolated from plants with developed symptoms and identified using the illustrated manual. A host range test was performed on plants in the glasshouse, as shown in Table 1, using the following method:

Four seeds from large-seeded and nine seeds from small-seeded plants were planted in 20-cm pots containing pasteurized soil. The inoculation of plants was performed as described for winter wild oat, in the glasshouse at 25/32 °C (day/night).

The response of plants to the pathogen was determined according to Abbas and Boyette (1992) and Abbas *et al*., (1991). The mortality rate of plants was also determined according to Abbas and Boyette (1992). In addition, thin layer chromatography was used to demonstrate toxin production by the fungus. The host range tests were carried out twice.

Fusarium spp. was isolated from all winter wild oat that exhibited disease symptoms. Among the 45 isolates, 94% were identified as *F. moliniforme,* 4% *F. lateritium,* and 2% *F. subglutinans.* The former isolate which was considered the main pathogen, grew rapidly on PDA (14mm/day at 24 °C). The pathogen produced salmon-colored hyphae and abundant microconidia, within 2-3 days on PDA. Macroconidia were produced at the centre of the culture media, which darkened with age. The fungus also grew well on autoclaved barley medium.

In experimental tests, *F.moniliforme* was highly virulent on *A. ludoviciana*. Among the three inoculation methods used, (a) was considered to be the best because it was simpler, more practical and uniform results were obtained in all replications.

Results of the host range test using method (a) are shown in Table 1. Winter wild oat, with 78% mortality, was considered very sensitive to the pathogen. Johnsongrass, crested wheatgrass and tomato, respectively with 19%, 24%, and 17% mortality were sensitive. Other test plants showed no symptoms. Certain

toxins were detected in the initial thin layer chromatography studies, identification of which will be subject of future studies. Taken together, it can be concluded that the host specificity of *F. moniliforme* makes it a potential biocontrol agent of winter wild oat in Iran.

Table 1. Response and mortality rate of test plants to *Fusarium moniliforme.*

Test plant	Reaction to *F. moniliforme**	Mortality rate (%)
Wheat (*Triticum aestivum* L.) var. Falat	R	0
Wheat (*Triticum aestivum* L.) var. Qods	R	0
Barley (*Hordeum vulgare* L.) var. Valfajr	R	0
Barley (*Hordeum vulgare* L.) var. Kavir	R	0
Maize (*Zea mays* L.) var. SC704	R	0
Winter wild oat (*Avena ludoviciana* Dur.)	VS	78
Rye (*Secale cereale* L.)	R	0
Johnsongrass [*Sorghum halepense* (L.) Pers.]	S	19
Millet [*Setaria italica* (L.) P. Beauv.]	R	0
Faba beans (*Vicia faba* L.) var. Barekat	R	0
Green beans (*Phaseolus vulgaris* L.) var.Top crop	R	0
Kidney beans (*Phaseolus vulgaris* L.) var. Naz	R	0
Sunflower(*Helianthus annuus* L.) var. Record	R	0
Soybeans [*Glycine max* (L.) Merrill] var. Williams	R	0
Oil seed Rape (*Brassica napus* L.) var. Ceres	R	0
Cotton (*Gossypium herbaceum* L.) var. Sahel	R	0
Safflower (*Carthamus tinctorius* L.) var. 2811	R	0
Tomato (*Lycopersicon esculentum* Mill.) var. Red cloud	S	17
Cucumber (*Cucumis sativus* L.) var.Dora F.	R	0
Water melon (*Citrulus vulgaris* Schrad.) var. Krimson sweet	R	0
Berseem clover (*Trifolium alexandrium* L.)	R	0
Sainfoin (*Onobrychis Verae* Sirj.) Karaj echotype	R	0
Crested wheatgrass [*Agropyrum desertorum* (fisch.) Schults]	S	24

* R= resistant (no visible symptoms); S= sensitive (either necrosis or stunting);
VS = very sensitive (Necrosis and stunting).

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