Proceedings of the Eighth International Symposium on Biological Control of Weeds
2-7 February 1992, Lincoln University, Canterbury, New Zealand.
Delfosse, E.S. and R.R. Scott (eds). DSIR/CSIRO, Melbourne, pp. 487-92 (1995).

Host Range and Evaluation of An Isolate of *Exserohilum turcicum* on Some Populations of Johnsongrass (*Sorghum halepense*)

P. Del Serrone[1] and L. Fornasari[2]

[1] Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero, 22-00156 Rome, Italy
***[2] European Biological Control Laboratory, U.S. Department of Agriculture-Agricultural Research Service, BP 4168-Agropolis, 34092 Montpellier* Cedex 5, France**

An isolate of *Exserohilum turcicum*, an endemic foliar pathogen of Johnsongrass (*Sorghum halepense*) collected in Italy was evaluated as a potential mycoherbicide. It was tested on 7 populations of the weed. The effect of various leaf wetness periods (6, 12, 24, and 32 h) and of the growing stages of plants (28, 45 and 55 d after germination) on infectivity of *E. turcicum* was determined under greenhouse conditions. The fungus requires at least 24 h of dew to induce disease on seedlings at all growing stages. *E. turcicum* killed up to 95% and reduced 88% of fresh weight of Johnsongrass plants when seedlings of 28 d after germination were inoculated. No significant variation in susceptibility among the populations of Johnsongrass tested was observed. The growing stage of the plant influenced the severity of the disease. The fungus induced lesions on 15 among the varieties of sorghum tested. Only 5 of them were considered susceptible to the pathogen. No disease reaction was recorded among 27 hybrids of maize tested. No symptoms appeared on any of the other plant species and varieties tested.

Introduction

Johnsongrass, *Sorghum halepense* (L.) Pers. (Graminaceae), a native of the Mediterranean region, is a serious perennial monocotyledonous weed worldwide (Holm *et al.* 1977).

Chemicals have been used to control Johnsongrass in Italy. More than 50 active ingredients have been registered against this weed. Some projects for the biological control of this weed have been investigated and many pathogens are being evaluated for their effectiveness and host-specificity (Chiang *et al.* 1989).

The efficacy of 30 fungal isolates, which were isolated from seeds and diseased Johnsongrass plants collected in Italy, was evaluated in 1990 (Del Serrone *et al.* 1990). An isolate of *Exserohilum turcicum* (Pass.) Leonard & Suggs warranted further investigation. Therefore, the objectives of this study were to determine efficacy of the isolate of *E. turcicum* against different populations of Johnsongrass and the infectivity of this pathogen on different varieties of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* [L.] Moench.) since it is reported in the literature as a pathogen of both these crops.

Methods and Materials

Fungal Isolation and Storage

Tissues cut from the diseased leaves were surface-sterilized in sodium hypochlorite (0.3%), rinsed in sterile distilled water and then plated on water agar (OXOID) in Petri dishes. Conidia developed from the lesions on the tissues were transferred to other Petri dishes containing 20 ml of Czapeck agar (OXOID) + 2% *V8* (Campbell©) juice which was the best culture medium among the several tested for production of conidia. The dishes were incubated at constant 20 and 30°C alternately (thermoperiod of 12 h) with 12 h photoperiod at near ultraviolet

(N.U.V.) light, (360 nm) at 30°C to induce conidia production.

Conidia were stored as follows: they were gently scraped from the lesions of infected leaves and suspended in water and *Tween 80* (0.1%). Disks of filter paper (5 mm dia.) were dipped in the conidial suspension, then dried for 24 h at 25°C, and stored at 5°C in dark (Luongo, Del Serrone & Fornasari, unpublished data).

Inoculum Production

Mycelium grown in liquid culture (Czapeck broth + 2% of *V8* juice) was encapsulated in sodium alginate matrix gelled in $Ca(Cl)_2$ (0.25 M) (Connick 1989). To obtain conidia, the granules containing fungal mycelium were placed in moistened filter paper in trays covered with a transparent plastic film. They were incubated at 25°C with N.U.V. light (photoperiod: 12 h) for 3 d. The plastic film was removed after 24 h. The dried granules were stored in plastic bottles at 20°C in dark.

Conidial suspensions, which were obtained by washing and stirring the granules in water and *Tween 80* (0.1%) solution, were sprayed to plants until runoff. An adhesivant was added to the conidial suspension before spraying.

Pathogenicity Tests

Johnsongrass seeds from 7 collections (Table 1) were sterilized and germinated under the following conditions: immersion in a solution of NaOCl (0.7 M) for 24 h; washing in sterile distilled water; disposition on a sandwich of cotton and filter paper moistened with KNO_3 (0.05 M) in plastic boxes; incubation at constant 20°C and 30°C alternately (thermoperiod 12 h) with continuous exposure to cool white fluorescent lamps (5 klux) for 7 d.

Table 1. Disease severity (D.S.)[1] of Johnsongrass plants from 7 different collections artificially inoculated with *Exserohilum turcicum* at 4 dew period treatments.

Collection		Dew Treatment (h)			
Initials[2]	Origin	6	12	24	32
OZZ	Northern Italy	0.08[3]	0.13	0.29	0.58
MCR	Central Italy	0.11	0.18	0.39	0.51
M 179	Mississippi (USA)	0.09	0.11	0.31	0.60
I 14	Iowa (USA)	0.10	0.18	0.21	0.59
WV 258	West Virginia (USA)	0.09	0.15	0.33	0.70
TM	Central Italy	–	0.20	0.40	0.55
ISPV	Southern Italy	–	0.18	0.37	0.53

[1] $D.S. = \frac{[\Sigma \text{ (Severity rating)(number of plants in the rating)}]}{\text{(Total number of plants)(highest severity rating)}}$

Rating levels: 0 = no infection; 1 = 0-20% infected leaf area (i.l.a.); 2 = 21-40% i.l.a.; 3 = 41-60% i.l.a.; 4 = 61-80% i.l.a.; 5 = 81-100% i.l.a. or dead plant. The disease index at or below 0.40 is considered resistant and that above 0.41 is considered susceptible.

[2] Initials consist of a 2-3 letter designation for country or region of origin.

[3] Average of 3 replicates each of 10 plants of 40 d after germination in 2 experiments.

Four dew period treatments of 6, 12, 24, and 32 h were evaluated. Plants of 40 d after germination (*D.A.G.*) were used for each seed collection. They were placed in transparent plastic boxes where dew was maintained with a saturated salt solution (Dinghra and Sinclair 1987) and artificially inoculated with a conidial suspension (10^4 conidia/ml) of *E. turcicum* under greenhouse conditions (20-25°C; 60-70% RH; 12 h photoperiod, Sylvania *Growth Lux*© lamps). Three replicates each of 10 plants each seed collection at each dew treatment . The experiment was replicated twice.

Disease severity (*D.S.*) was rated 14 d after inoculation using 6 rating levels: 0 = no infection; 1 = 0-20% infected leaf area (i.l.a.); 2 = 21-40% i.l.a.; 3 = 41-60% i.l.a.; 4 = 61-80%

i.l.a.; 5 = 81-100% or dead plant. A disease score was then calculated from the ratio of:

$$\frac{[\Sigma \text{ (Severity rating)(no. of plants in the rating class)}]}{\text{(Total no. of plants)(highest severity rating class)}}$$

The influence of 3 growing stages (28, 45, and 55 *D.A.G.*) on *D.S.* was determined 14 d after inoculation (10^6 conidia/ml) as percentage of mortality and fresh weight reduction. The experiment was conducted under the same greenhouse conditions with a 32 h dew treatment. Three replicates each of 10 plants were considered for each seed collections and growing stages considered. Averages obtained were statistically compared using the Newman-Keuls Student's test.

Host-Specificity Tests

The fungus was artificially inoculated (10^6 conidia/ml) under greenhouse conditions to 40 varieties of cultivated sorghum, 25 hybrids of maize and 5 varieties for each of the following crops: durum wheat, bread wheat, oat, barley and rice. American varieties of sweet corn, millet and rye were also tested (Table 2).

The test was preliminarily carried out by artificial inoculation of detached leaves on filter paper moistened with a Kinetin solution (0.5%) in Petri dishes. Then whole plants at 3 different growing stages (first, second and third unrolled leaf) were used. Each thesis had 3 replications and each replication had 10 plants. They were maintained in the greenhouse as previously mentioned. The experiment was replicated twice. The *D.S.* was rated as indicated above 32 h after dew treatment.

Production of conidia on randomly selected detached leaves was recorded under the stereomicroscope after 24 and 48 h of incubation in moist chamber. The following scale was used: – no conidia produced; + few conidia produced; ++ moderate to heavy conidia production.

Results

Inoculum Production

Conidia produced from mycelium encapsulated in granules of sodium alginate were still pathogenic 1 yr after preservation under the above mentioned conditions. Conidia (up to 3.6 x 10^6 conidia/ml) could be obtained 3 times by suspending the same granules (1 g) in 15 ml of water and *Tween 80* solution.

Pathogenicity Tests

The treatment of Johnsongrass seeds allowed standardization of plant production for the tests with about 80% germination after 7 d and 100% seedling rooting.

The fungus produced necrotic spots which spread on the blade of the leaf 24 h after inoculation. These spots became elliptical lesions 2-3 d later and subsequently they enlarged and coalesced.

Johnsongrass plants of 40 *D.A.G* which were inoculated and incubated in dew boxes for 32 h were highly susceptible to the fungus (D.S. >0.5). Some plants were also killed (Table 1).

Furthermore the mortality of Johnsongrass plants was increased considerably (up to 95%) when the fungus was artificially inoculated to seedlings of 28 *D.A.G.* The pathogen reduced the fresh weight up to 88% when the seedlings of 28 *D.A.G.* were inoculated (Table 3).

There was no significant difference in the mortality and fresh weight reduction among the 7 Johnsongrass populations tested.

Host-Specificity Tests

Sorghum proved to be compatible with the fungus. Among the 40 varieties of sorghum tested, 15 showed symptoms. The hypersensitive or susceptible reaction of the host/pathogen compatible systems was recorded after 48 h of incubation in moist chamber of detached leaves randomly cut from whole artificially inoculated plants. At that time moderate to heavy conidia production was recorded only on 5 varieties (*D.S.* range 0.27-0.37). They are therefore considered susceptible. The average disease index recorded at the 3 growing stages, was never higher than 0.37. There was no difference among the scores recorded at the different growing stages.

Table 2. Disease severity (D.S.)[1], conidia production (C.P.)[2], and plant reaction (P.R.)[3] induced by *Exserohilum turcicum* on detached leaves of several cultivars of cereal crops under controlled conditions (20-25°C; 32 h dew treatment).

Test Plant	D.S.	C.P	P.R.	Test Plant	D.S.	C.P	P.R	Test Plant	D.S.	C.P.	P.R.
Sorghum				**Maize**				**Durum Wheat**			
"Arcadie"	0.03	*	i	"Derek"	0.00	*	i	"Capeiti"	0.00	*	i
"Arlequin"	0.00	*	i	"Meryl"	0.00	*	i	"Valnova"	0.00	*	i
"Esquirol"	0.00	*	i	"Mistral"	0.00	*	i	"Appulo"	0.00	*	i
"Poggio"	0.21	–	h	"Plauto"	0.00	*	i	"Creso"	0.00	*	i
"Rubus"	0.00	*	i	"Sting"	0.00	*	i	"Trinakria"	0.00	*	i
"Arno"	0.00	*	i	"Strato"	0.00	*	i				
"Arabella"	0.00	*	i	"Valeria"	0.00	*	i	**Bread Wheat**			
"Aragon"	0.18	–	h	"Zia"	0.00	*	i	"Pandas"	0.00	*	i
"Ardan"	0.00	*	i	"Rosso"	0.00	*	i	"Farneto"	0.00	*	i
"Citrus"	0.00	*	i	"Aida"	0.00	*	i	"Ariano"	0.00	*	i
"Dallas"	0.00	*	i	"Alibert"	0.00	*	i	"Orso"	0.00	*	i
"Ginepro"	0.00	*	i	"Arco"	0.00	*	i	"Manital"	0.00	*	i
"NK121"	0.15	–	h	"Ciclone"	0.00	*	i	"Borah"[4]	0.00	*	i
"Prunus"	0.08	–	h	"Congo"	0.00	*	i				
"Sultano"	0.07	–	h	"Franca"	0.00	*	i	**Oat**			
"Vico"	0.00	*	i	"Larch"	0.00	*	i	"Ava"	0.00	*	i
"Alabama"	0.00	*	i	"Leone"	0.00	*	i	"Ombrone"	0.00	*	i
"Anatol"	0.00	*	i	"Licinio"	0.00	*	i	"Kallott"	0.00	*	i
"Argelo"	0.32	+	s	"Look"	0.00	*	i	"Angelica"	0.00	*	i
"Cactus"	0.27	+	s	"Mirto"	0.00	*	i	"Argentina"	0.00	*	i
"Dakota"	0.33	++	s	"Nelson"	0.00	*	i	"Otana"[4]	0.00	*	i
"Excelsior"	0.28	+	s	"Publio"	0.00	*	i				
"Gran Sasso"	0.00	*	i	"Sirena"	0.00	*	i	**Barley**			
"Hazera"	0.00	*	i	"Stiff"	0.00	*	i	"Protidor"	0.00	*	i
"Manol"	0.17	–	h	"Giovanna"	0.00	*	i	"Pirate"	0.00	*	i
"Minotauro"	0.27	–	h	"Roberta"	0.00	*	i	"Fiction"	0.00	*	i
"Monteverde"	0.00	*	i	"A632/A619"[4]	0.00	*	i	"Kaskade"	0.00	*	i
"Nevada"	0.37	*	i					"Tipper"	0.00	*	i
"NK180"	0.00	*	i	**Sweet Corn**				"Kristal"	0.00	*	i
"Proton"	0.00	*	i	"49261/cr19"[4]	0.00	*	i				
"Taxus"	0.00	*	i					**Millet**			
"Tean"	0.00	*	i	**Rice**				"Tifleaf"[4]	0.00	*	i
"Aralba"	0.00	*	i	"Balilla"	0.00	*	i				
"Argence"	0.00	*	i	"Elio"	0.00	*	i				
"Arianna"	0.00	*	i	"Cripto"	0.00	*	i				
"Consol"	0.00	*	i	"Lido"	0.00	*	i				
"Cardus"	012	–	h	"Argo"	0.00	*	i				
"Castoro"	0.21	–	h								
"Dorado E"	0.34	+	s								
"Lorenzo"	0.00	*	i								
"IS7173C"[4]	0.00	*	i								

[1] Recorded on plants of 3 growing stages (1st, 2nd, 3rd unrolled leaf) artificially inoculated with *Exserohilum turcicum* (10^5 conidia/ml) in controlled conditions (20-25°C; 32 h dew treatment).

[2] Recorded on detached leaves, randomly chosen, with symptoms after 24 and 48 h. Scale: – = no conidia produced; + = few conidia produced; ++ = moderate to heavy conidia produced.

[3] Scale: i = immune; h = hypersensitive; s = susceptible; and * = absence of symptoms (fungus never reisolated).

[4] American varieties tested only at 2nd unrolled leaf.

Table 3. Percentages of mortality (M) and fresh weight reduction (FWR) of Johnsongrass populations of different days after germination (*D.A.G.*) artificially inoculated with *Exserohilum turcicum* in dew boxes for 32 h at 20-25°C.

Collection	*D.A.G.*					
	28		45		55	
	M%[1]	FWR %[2]	M%	FWR %	M%	FWR %
OZZ	90 *Aa*	81 *Aa*[3]	50 *Aa*	15 *Aa*	26 *Aa*	9 *Aa*
MCR	90 *Aa*	80 *Aa*	47 *Aa*	20 *Bb*	30 *Cc*	8 *Aa*
M 179	95 *Ba*	88 *Ba*	50 *Aa*	20 *Bb*	20 *Ba*	8 *Aa*
I 14	90 *Aa*	80 *Aa*	44 *Cc*	12 *Aa*	30 *Cc*	9 *Aa*
WV 258	91 *Aa*	88 *Aa*	60 *Bb*	29 *Cc*	31 *Cc*	10 *Aa*
TM	89 *Aa*	80 *Aa*	58 *Bb*	43 *Cb*	31 *Cc*	10 *Aa*
I SPV	87 *Ba*	81 *Aa*	50 *Aa*	18 *Bb*	27 *Aa*	7 *Aa*

[1]Average of 3 replicates each of 10 plants in 2 experiments.
[2]Average of 15 plants randomly chosen in 2 experiments.
[3]Average within the rows followed by the same letter are not significantly different (Newman-Keuls Student's test; $P \geq 0.01$ upper case, $P \geq 0.05$ lower case).

No disease reaction was recorded on the 27 hybrids of maize and on the other several varieties of crops tested (Table 3). These results confirmed those obtained with the screening *in vitro* on detached leaves.

Conclusions

The fungus can induce disease on Johnsongrass plants at different growing stages. As shown in Table 1 the length of the dew period increases the damage caused by the pathogen. With a 32 h dew period treatment the frequency of 5th rating level was higher and some plants died. Different populations of the weed show a different degree of reaction and Johnsongrass plants from West Virginia were the most susceptible.

This isolate of *E. turcicum* when inoculated on seedlings of Johnsongrass with 32 h dew period and 10^6 conidia/ml killed >90% plants of all the different populations of Johnsongrass of 28 *D.A.G.* The need for a long dew period is not a constraint for using this fungus under field conditions. There are several reports in the literature of adjuvants that can be used for reducing the evaporation of water in which generally conidia or spores are suspended (Amsellem *et al.* 1990, Daigle *et al.* 1990).

Conidia can be easily obtained under appropriate conditions. Furthermore the fungus is highly pathogenic and shows a restricted host range. Only cultivated sorghum is compatible among the several crops tested against the fungus. However, the fungus is a very weak pathogen of this crop even though inoculated under the optimum conditions that induce mortality of its natural host. The availability of varieties of sorghum that are not susceptible with the fungus will allow selection of those that can be safely cultivated in field where this fungus would be used as a mycoherbicide.

The isolate of *E. turcicum* tested in this study seems to be the most promising for biological control of Johnsongrass, among the isolates tested up to date. Nevertheless field studies will still be necessary to further evaluate its real potential as mycoherbicide.

Acknowledgments

We thank Dr. L.A. Lacey, Dr. L. Knutson, Prof. A. Quacquarelli and Dr. S.M. Yang for reviewing the manuscript. We are also grateful to Dr. G.A. White for providing American seeds of cultivated plants and of Johnsongrass for testing.

References

Amsellem, Z., A. Sharon, J. Gressel and P.C. Quimby, Jr. 1990. Complete abolition of high inoculum threshold of two mycoherbicides (*Alternaria cassiae* and *A. crassa*) when applied in invert emulsion. *Phytopathology* **80**:925-9.

Chiang, M.Y., C.G. Van Dyke, and K.Y. Leonard. 1989. Evaluation of endemic foliar fungi for potential biological control of Johnsongrass (*Sorghum halepense*): screening and host range tests. *Plant Disease* **73(6)**:459-64.

Connick, W.J. Jr. 1989. Formulation of living biological control agents with alginate. *In*: *Pesticides formulations: Innovations and developments.* Barrington Cross and H.B. Scher (eds.). *ACS Symposium Series No.* **371**:241-50.

Daigle, D.J., W.J. Connick, Jr., P.C. Quimby, Jr. J. Evans, B. Trask-Morrell and F.E. Fulgham. 1990. Invert emulsions: carrier and water source for the mycoherbicides, *Alternaria cassiae. Weed Technology* **4**:327-31.

Del Serrone, P., S. Lucchesi, E. Forti and A. Quacquarelli. 1990. Screening and pathogenicity tests of fungi isolated from Johnsongrass. *In*: *Proceedings of the Symposium on Pesticides and Alternatives*, Kolymbari Crete and J. Casida (eds.). Elsewier, 365 p.

Dinghra, O. D. and J. B. Sinclair. 1987. *Basic Plant Pathology Methods.* CRC Press, 355 p.

Holm, L.G., D.L. Plucknett, J.V. Pancho, and J.P. Herberger. 1977. *The World's Worst Weeds: Distribution and Biology.* University Press of Hawaii, Honolulu, 609 p.