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Plant pathology

Pathogenicity of *Fusarium* spp. Incidence on soybean seed quality

G.A. Lori[1] and S.J. Sarandon[2]

[1] *Catedra de Fitopatologia*
[2] *Catedra de Cerealicultura, Facultad de Agronomia, Universidad Nacional de La Plata, 60 y 118, (1900) La Plata, Buenos Aires, Argentina*

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Summary — *Fusarium semitectum* Berk & Rav. has been often cited as causal agent of soybean (*Glycine max* (L.) Merril) pod rot. Eighteen strains of different species belonging to the same genus (*F. equiseti* (Corda) Sacc., *F. fusarioides* (Frag. & Cif.) Booth, *F. graminearum* Schwabe, *F. moniliforme* Sheldon, *F. sambucinum* var. *coeruleum* Fuckel Wollenw., *F. semitectum* and *F. solani* (Mart.) Sacc.) isolated from soybean seeds were inoculated on soybean pods on the plant, wounded and unwounded, at developing stages $R_{5,5}$ and $R_{6,5}$ to verify if their pathogenic capacity was identical to *F. semitectum* and to determine their incidence on injury frequency and seed quality according to the time of attack. Injury frequency was registered 20 and 40 days after inoculation. The two observation dates showed higher injury frequency on wounded pods for both developmental stages. On day 20, injuries were higher when inoculation had been performed at $R_{5,5}$. However, difference rates were less on day 40. Hundred seed weight was more affected at $R_{5,5}$ than at $R_{6,5}$. Germinative capacity was not greatly affected at both reproductive stages in unwounded pods; but it was almost non-existent in previously wounded pods.

The frequent presence of *F. semitectum* is not attibuted to its particular pathogenicity, but to its capacity for widespread distribution. It should be pointed out that the method applied here may not resemble the processes in nature and that these artificial conditions may have favoured the development of certain species to the detriment of others.

soybean seeds – *Fusarium* pathogenicity – developing stages – seed quality – pod rotting

Résumé — Pouvoir pathogène d'espèces du genre fusarium et incidence sur la qualité des graines de soja. Fusarium semitectum *Berk et Rav. est cité communément comme agent de la pourriture des gousses de soja (*Glycine max*) (L.) Merril). Afin de vérifier si d'autres espèces du genre* Fusarium *isolées des graines de soja possèdent le même pouvoir pathogène et déterminer leur incidence selon le moment d'attaque dans la fréquence des dégâts et la qualité des graines, on a inoculé 18 échantillons de différentes espèces* (F. equiseti (*Corda*)., F. fusarioides (*Frag. & Cif*) *Booth*, F. graminearum *Schwabe*, F. moniliforme *Sheldon* F. sambucinum *var.* coerulum *Fuckel Wollenw.*, F. semitectum et F. solani) *sur des plantes de soja, avec ou sans blessures des gousses, au stade de développement* $R_{5,5}$ et $R_{6,5}$.

La fréquence des attaques est déterminée 20 et 40 jours après inoculation. Aux deux stades et aux deux dates d'observation, on note le plus fort pourcentage d'attaque sur gousses blessées. Au 20e jour, on observe que les attaques sont les plus fortes lorsque les inoculations ont été effectuées au stade $R_{5,5}$. *Par contre, au 40e jour, les différences sont moindres. Au stade* $R_{5,5}$ *le poids de 100 grains est plus affecté qu'au stade* $R_{6,5}$. *le pouvoir germinatif aux deux stades n'est pas très affecté si les semences proviennent de gousses saines, mais il est presque nul pour des semences venant de gousses blessées. Sous les mêmes conditions d'inoculation, toutes les espèces se comportent de façon similaire. Malgré la pénétration du mycelium au moment de la fructification, les semences de gousses non blessées montrent un bon pouvoir germinatif, quelle que soit la souche. La présence des* Fusarium *ssp. sur les graines est plus importante lorsque les champignons sont entrés en contact avec les gousses dès le stade le plus précoce du développement (*$R_{5,5}$*).*

On conclut que si F. semitectum *est trouvé le plus fréquemment sur le soja, ce n'est pas dû principalement à un pouvoir pathogène particulier, mais plutôt à son aptitude à se disperser facilement. Notons que les techniques utilisées peuvent être différentes des processus naturels et que les conditions artificielles peuvent avoir favorisé certaines espèces.*

graines de soja – pouvoir pathogène – stade de développement – qualité des graines – pourriture des gousses

Introduction

Research works carried out on soybean (*Glycine max* (L.) Merril) seed pathology has distinguished 9 contaminating fungi genera. Of these, only *Phomopsis* and *Fusarium* were connected with reduction in seed viability (McGee, 1981).

Several species of *Fusarium* have been cited as being causal agents of soybean seed quality loss (Neergaard, 1983) whereas the pathogenic nature has not been established. *Fusarium* spp. incidence increases when harvesting is delayed (Dhingra *et al.*, 1978; Hepperly and Sinclair, 1981). *Fusarium semitectum* Berk & Rav. is commonly associated with poor health in soybean seeds (Dhingra *et al.*, 1978; Hepperly and Sinclair, 1981). In India it has been reported as causal agent of pod and collar rot (Saharan and Gupta, 1972). According to Dhingra *et al.* (1978), the fungus can penetrate through the uninjured pod walls and infect the seeds; however, the developmental stage at which the attack appears has not been determined.

In previous assays, several *Fusarium* species other that *F. semitectum* were observed to prevent germination at different frequencies. Several species existing in the seeds might possibly have an identical pathogenic capacity and, as *F. semitectum*, affect quality, produce infection during the developmental stages and penetrate pod walls.

The aim of this work was to test the pathogenicity of different *Fusarium* spp. on soybean plants at different growth stages, to detect the penetrability and to determine strain attack incidence on injury frequency and seed quality.

Materials and Methods

Eighteen strains, obtained from single spore cultures belonging to 7 species, were selected for inoculation: *Fusarium equiseti* (Corda) Sacc. (5 strains), *F. fusarioides* (Frag. & Cif.) Booth (1 strain), *F. graminearum* Schwabe (1 strain), *F. moniliforme* Sheldon (2 strains), *F. sambucinum* var. *coeruleum* Fuckel Wollenw. (2 strains), *F. semitectum* (4 strains) and *F. solani* (Mart.) Sacc. (3 strains). All of them were isolated from contaminated soybean seeds with lack of viability. Booth's (1971) system of classification and description was used to identify the species.

Cultivar Williams (maturity group III) was used; the plants were obtained from seeds disinfected with 5% sodium hypochlorite (NaOCl) for 5 min and rinsed with deionized sterile water. They were sown in pots with autoclaved soil, and kept in a glasshouse until they reached $R_{5,5}$ (mid-pod filling) and $R_{6,5}$ (full-pod before physiological maturity) (Fehr and Caviness, 1977). Silhouette method was applied to detect seeds' real developmental stage, *i.e.* to observe fruit seed-size through daylight (Spaeth and Sinclair, 1984). Treatments consisted of inoculating each strain into healthy and wounded pods, distinctly identified, at both growth stages ($R_{5,5}$ and $R_{6,5}$). Lesions were made with sterile needle (0.3 mm diameter) on each fruit seed.

To prepare inoculum each of the strains was cultured in Petri dishes with PDA (potato–dextrose–agar), and incubated at 20°C (± 2°C) for a week in chambers with near ultra violet light (NUV) to induce sporulation.

Spores were suspended in deionized sterile water and spore concentration was adjusted to $1 \cdot 10^5$ spore (macro or macro and microconidia)/ml for each strain. The suspensions were sprayed over the plants until soaking. In the controls, pods were sprayed with deionized sterile water. All the plants were kept in humid chamber for 48 h.

Records were taken daily for abortion, necrotic pods and rotting and data were processed on day 20 and on day 40 after inoculation to determine injury frequency (injured pod/inoculated pod · 100).

Hundred seed weight, germinative capacity and *Fusarium* spp. presence were determined for every treatment. Seeds were sown in Petri dishes with two wet filter papers and incubated at 20°C in a chamber with NUV light. Seven days after sowing, germinative capacity was checked and *Fusarium* presence, either by mycelium or by pionnotes from the strains inoculated, was registered on the seeds.

The assay followed a completely random design with 3 replications, with 20 subsamples (pods) per experimental unit. Variance analysis following a factorial pattern was used for the analysis of the data; strain and lesion presence or absence on the pods were considered to be factors. Averages were compared by Tukey's test ($P = 0.05$).

Results

Symptoms observed

The species and inoculated strains showed no differential behaviour as regards nature of the infection.

Stage $R_{5,5}$

When wounded pods were inoculated, symptoms began at day 6. These was darkening and necrosis of the tissue encircling the lesion spot, with circular expansion until drying the whole pod. Pod growth stopped and consequently, there was a high pod abortion rate within 20 days of inoculation.

When there was no previous lesion, few strains produced distinct symptoms within the same period. Injury was manifested later through dry rot

that appeared on ventral bundles and expanded over the whole pod surface. In the fruits, seeds were found to be wrinkled, small-sized, dried and blackened.

Stage $R_{6,5}$

Pods that been previously wounded presented seed darkening, visualized through pod walls, with later rotting of the fruit.

In the unwounded treatment, the low number of pods affected presented dry rot; its onset was similar to the stages ($R_{5,5}$). Seeds were affected with rot symptoms.

Data analysis

There were differences between the strains used, reproductive stages at which inoculation was performed, and the times at which observations were recorded. There was significant interaction between inoculated strains and pod treatments for all the parameters tested (Table I).

Injury frequency (Table I)

Injury frequency between wounded and unwounded treatments varied in the reproductive stages and observation dates. The highest degree of injury was found in previously wounded pods; 20 days after inoculation injury frequency was higher for $R_{5,5}$ (77.2%) than for $R_{6,5}$ (33.5%). However, rate difference was less when it was recorded at day 40, 93.3 and 87.4%, respectively.

Twenty days after inoculation, unwounded pods presented low injury frequency for both reproductive stages. On the contrary, this increased on day 40, affecting pods inoculated at the earlier stage.

Table I. Injury frequency registered 20 and 40 days after inoculating unwounded and wounded soybean (cv Williams) pods at developmental stages $R_{5,5}$ and $R_{6,5}$.

Strains	*Injury frequency (%)*							
	$R_{5,5}$				$R_{6,5}$			
	20 days		*40 days*		*20 days*		*40 days*	
	unwounded	*wounded*	*unwounded*	*wounded*	*unwounded*	*wounded*	*unwounded*	*wounded*
Control	0.0	0.6	0.0	4.0	0.0	0.0	0.0	0.0
F. equiseti	0.0	93.0	31.0	99.0	0.4	74.0	0.3	95.4
F. equiseti	1.7	88.0	5.0	98.0	0.0	13.0	5.6	91.0
F. equiseti	0.0	81.0	2.0	99.0	0.0	29.0	3.2	99.7
F. equiseti	0.0	61.0	5.0	99.0	0.0	25.0	1.3	96.7
F. equiseti	0.0	91.0	39.0	92.0	0.0	92.0	4.6	98.6
F. fusarioides	13.0	98.0	39.0	100.0	0.0	32.0	1.9	90.3
F. graminearum	2.0	91.0	3.0	100.0	0.0	75.0	16.0	100.0
F. moniliforme	0.0	94.0	19.0	98.0	0.0	76.0	0.0	99.6
F. moniliforme	0.0	92.0	26.0	100.0	0.0	67.0	9.0	97.6
F. samb. var. *coeruleum*	0.7	75.0	3.0	100.0	2.3	68.0	9.2	97.0
F. samb. var. *coeruleum*	0.0	65.0	12.0	98.0	0.4	9.0	9.4	80.3
F. semitectum	0.3	63.0	16.0	88.0	0.0	21.0	10.4	96.2
F. semitectum	0.7	64.0	16.0	99.0	0.0	0.0	1.6	88.0
F. semitectum	0.5	75.0	12.0	100.0	0.0	0.0	0.0	77.6
F. semitectum	23.0	96.0	28.0	100.0	0.0	12.0	10.6	67.6
F. solani	0.0	71.0	4.5	100.0	0.0	2.0	3.3	99.3
F. solani	5.4	100.0	11.0	100.0	0.0	40.0	9.2	97.5
F. solani	0.0	69.0	4.0	98.0	0.0	2.0	7.6	88.9
Average	2.5	77.2	14.5	93.3	0.2	33.5	5.4	87.4
LSD 5% (1)	3.41		4.10		9.30		8.60	
LSD 5% (2)	6.27		7.50		17.20		15.90	
Interaction strain x treatment	**		**		**		**	

(1) Comparison of treatments within each strain.
(2) Comparison of strains within each treatment.

Pathogenicity differences in the 18 strains manifested distinctly when the unwounded pods were inoculated at $R_{5,5}$ and when the observations were recorded 40 days later. Uniformity as regards pathogenicity could not be found within strains belonging to the same species.

Fusarium equiseti, *F. fusarioides*, *F. moniliforme* and *F. semitectum* were the strains that caused injuries >20%. Injury detected in the control wounded pods was caused by *Alternaria* sp.

Effects on hundred seed weight of the different strains

The seed weight seemed to be lower and was more affected when inoculation was performed at an earlier stage after a lesion (Table II).

Seed germinative capacity

In the wounded pods, this was significantly lower than in the unwounded; it decreased when inoculation was performed at $R_{5,5}$ (Table III). As regards *Fusarium* spp., wounded pods showed higher percentages for both inoculation stages.

Although unwounded pods had better germinative capacity, hyphae penetrated through fruit walls and invaded the seeds; the attack was stronger at the earlier inoculation stage (Table III).

Discussion

When *Fusarium* spp. penetrates the pods through lesions made purposefully during fruit seed filling,

Table II. Soybean (cv, Williams) hundred seed weight, obtained from unwounded and wounded pods inoculated with *Fusarium* spp. at $R_{5,5}$ and $R_{6,5}$.

Strains	*Hundred seed weight (g)*			
	$R_{5,5}$		$R_{6,5}$	
	unwounded	*wounded*	*unwounded*	*wounded*
Control	19.2	16.2	19.4	17.5
F. equiseti	8.2	3.0	17.8	4.7
F. equiseti	7.2	2.1	14.4	3.3
F. equiseti	10.0	6.2	15.7	7.3
F. equiseti	9.1	4.8	18.5	5.9
F. equiseti	8.2	2.0	15.1	4.4
F. fusarioides	6.6	0.5	15.1	6.6
F. graminearum	8.0	2.0	12.9	3.1
F. moniliforme	13.0	2.5	17.8	3.6
F. moniliforme	12.2	1.7	15.5	3.2
F. sambucinum var. *coeruleum*	12.6	1.7	12.8	4.6
F. sambucinum var. *coeruleum*	11.1	4.0	15.8	4.4
F. semitectum	10.5	2.3	17.0	5.3
F. semitectum	11.1	2.1	17.0	7.0
F. semitectum	10.0	5.0	18.0	7.0
F. semitectum	8.6	1.5	13.6	5.4
F. solani	7.8	3.1	17.0	4.3
F. solani	11.7	0.0	9.6	3.5
F. solani	10.9	6.7	15.7	7.4
Average	10.3	3.5	15.9	5.7
LSD 5% (1)	0.98		0.82	
LSD 5% (2)	1.82		1.52	
Interaction strain x treatment	**		**	

(1) Comparison of treatments within each strain.

(2) Comparison of strains within each treatment.

Table III. Seed germinative capacity and presence of *Fusarium* spp. on soybean (cv Williams) seeds from unwounded and wounded pods inoculated at $R_{5,5}$ and $R_{6,5}$.

Strains	*Germinative capacity (%)*				*Fusarium presence (%)*			
	$R_{5,5}$		$R_{6,5}$		$R_{5,5}$		$R_{6,5}$	
	unwounded	*wounded*	*unwounded*	*wounded*	*unwounded*	*wounded*	*unwounded*	*wounded*
Control	96.0	91.0	97.0	92.0	0.0	0.0	0.0	0.0
F. equiseti	82.1	2.0	94.9	0.0	41.1	96.3	9.6	100.0
F. equiseti	85.4	0.0	90.0	0.0	31.5	100.0	19.1	100.0
F. equiseti	86.8	0.0	92.6	0.0	30.3	100.0	20.6	99.3
F. equiseti	89.3	1.3	95.3	0.6	18.8	99.3	24.3	100.0
F. equiseti	82.1	2.0	91.0	0.3	41.2	98.6	10.0	100.0
F. fusarioides	62.4	0.0	96.6	0.3	63.0	100.0	25.7	100.0
F. graminearum	83.0	0.0	75.6	0.0	32.8	100.0	26.8	100.0
F. moniliforme	79.6	0.0	94.6	0.0	60.3	100.0	6.8	100.0
F. moniliforme	71.4	0.0	71.9	0.0	54.6	100.0	13.9	100.0
F. samb. var. *coerul.*	97.3	0.0	83.3	1.3	31.0	99.3	8.8	100.0
F. samb. var. *coerul.*	81.3	0.0	86.4	6.0	19.3	97.6	24.8	91.0
F. semitectum	89.2	9.7	88.9	7.0	3.5	62.8	12.0	100.0
F. semitectum	80.8	2.6	92.0	4.0	36.6	96.3	15.7	99.3
F. semitectum	83.1	0.0	93.3	3.3	33.6	99.3	16.5	98.6
F. semitectum	68.8	0.0	84.7	22.2	80.0	100.0	17.5	86.2
F. solani	80.5	1.0	93.6	0.0	2.1	72.8	20.0	100.0
F. solani	85.8	0.0	74.9	0.0	2.3	100.0	24.3	100.0
F. solani	82.4	0.6	91.0	1.0	42.2	100.0	14.2	100.0
Average	82.5	5.8	88.8	7.3	32.8	90.6	16.3	93.4
LSD 5% (1)	3.65		4.51		4.62		4.37	
LSD 5% (2)	6.73		8.30		8.52		8.04	
Interaction strain x treatment	**		**		**		**	

(1) To compare treatments within each strain.
(2) To compare strains within each treatment.

its pathogenic capacity causes cessation of growth and, by affecting the embryo, pod abortion. Natural abortion, usually present in soybean and other legumes, had no incidence in this work because the reproductive stages ($R_{5,5}$ and $R_{6,5}$) assayed were after "final seed abortion stage" which, according to Pigeaire *et al.* (1986) begins at R_5 (Fehr and Caviness, 1977).

In this experiment, the highest injury percentage on artificially wounded pods indicates that lesions caused by other crop pests (Kilpatrick and Hartwig, 1955), such as the southern green stink bug, *Nezara viridula* L., could facilitate *Fusarium* spp. penetration and contribute to fruit abortion and seed loss during fructification.

If *Fusarium* spp. infect pods early during fructification, pod rot increases and seed quality diminishes. This might be attributed to the length of time during which the fungi is in contact with the fruit.

Soil particles, carriers of *Fusarium* spp. propagules that would naturally cover the pods, could increase inoculum density due to the colonization of the tissues (Schlub *et al.*, 1981) and favour pathogenesis.

In the same manner as *Fusarium semitectum* (Saharan and Gupta, 1972), the other species, inoculated after being isolated from seeds, showed their pathogenic capacity, causing pod rot and penetrating through unwounded walls during pod filling.

When the species invades pods at the last stage in fructification ($R_{6,5}$), injury is lower. The short period of contact with the fruit could hinder fungi development and lessen pathogenic effect, as has already been described for *Phomopsis* spp. (Rupe and Ferris, 1986). Seed quality is less affected at this stage, as penetration occurred after seeds had accumulated the highest percentage of dry matter.

The increase in seed number infected by *Phomopsis* and *Fusarium* is often associated with humid periods following physiological maturity

(Spilker *et al.*, 1981), with greater seed losses as harvesting is delayed (Dhingra *et al.*, 1979). However, Rupe and Ferris (1986) demonstrated *Phomopsis* ability to develop and infect seeds over a wide water potential range. Seed infection occurs when the environmental conditions maintain pod water content below 19%; growth of several microorganisms is thereby restricted.

The high presence of *Fusarium* in unwounded pods at both reproductive stages ($R_{5,5}$ and $R_{6,5}$) would demonstrate that its penetration is possible since fructification. Fresh seeds revealed the presence of the different species inoculated, but seed germinative capacity was not greatly affected. This suggests that if harvest was delayed, injuries would increase because the fungi present in the fruit would increase inoculum density, as happens in soils with low matric potential (Schlub *et al.*, 1981).

Species of this genus might be pathogenic under severe water stress conditions, as was found for *Fusarium moniliforme* in maize (Schneider and Pendery, 1983). *Fusarium* spp. can obtain water for their germinating chlamydospores from soils with low matric potential, and also grow upon plant tissues with as low an osmotic potential as mature wheat (Cook and Christen, 1976).

All the species presented identical behaviour under equal inoculating conditions; there was no differential pathogenic capacity. In consequence, it can be concluded from these results that frequent presence of *F. semitectum* in soybean could not be attributed to physiological differences as regards pathogenic nature. It should be pointed out that the method applied here may not resemble the processes developed in nature. Also, these artificial conditions might have favored species not frequently isolated from soybean pods. Consequently, it became difficult to distinguish the pathogenic capacity of *F. semitectum* from the other species capacity.

Nevertheless, there are other specific characteristics of *F. semitectum* that may contribute to its pathogenic capacity such as its widespread distribution in different climates and soils plus the production of dry-nature blastospores which aid air dispersion (Booth, 1971).

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