

***Fusarium* spp from potato: polymorphism of esterase phenotypes on polyacrylamide gel electrophoresis**

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(Received 29 June 1992; accepted 26 April 1993)

Summary — Polyacrylamide gel electrophoresis was used to analyze 49 single-spore cultures of *Fusarium* from potatoes growing in the Brittany region of France. The isolates belonged to 3 species (*F oxysporum*, *F solani*, and *F roseum*), 5 varieties of *F roseum* (*sambucinum*, *arthrosporioides*, *culmorum*, *graminearum* and *gibbosum*). Esterase patterns from the mycelium made it possible to unambiguously distinguish each species or variety. Depending on the species or varieties 6 to 20 isozymes were found, but only 3 or 4 of these could be used for identification. *F oxysporum*, *F solani* var *coeruleum*, and *F roseum* var *arthrosporioides* showed highly distinctive enzyme patterns, while the esterase patterns of *F roseum* var *culmorum* and var *graminearum* were closely related. Where morphological heterogeneity appeared between isolates of the same species or variety, the enzyme patterns were variable, such as in *F roseum* var *graminearum* with 2 stet morphological sub-types and in *F roseum* var *arthrosporioides* with its morphological variants. Based on these stable and fluctuating isozymes, we consider that *F roseum* var *arthrosporioides* and *F roseum* var *avenaceum* could be one and the same species. Lastly, the relation between tolerance to thiabendazole and esterase patterns of *F roseum* var *sambucinum* is discussed.

electrophoresis / isozymes / systematics / morphology

Résumé — Polymorphisme des isoenzymes d'estérases d'espèces ou de variétés de *Fusarium* isolées de tubercules de pomme de terre. L'électrophorèse sur gel de polyacrylamide est utilisée pour analyser 49 isolats monospores de *Fusarium* issus pour la plupart de pomme de terre en Bretagne. Ces isolats appartiennent à 3 espèces (*F oxysporum*, *F solani* et *F roseum*), et 5 variétés de *F roseum* (*sambucinum*, *arthrosporioides*, *culmorum*, *graminearum*, *gibbosum*). Les estérases du mycélium, seul critère enzymatique retenu, permettent de distinguer sans ambiguïté chaque espèce ou variété de *Fusarium*. Selon les espèces ou les variétés, 6 à 20 bandes enzymatiques sont mises en évidence mais 3 à 4 seulement peuvent servir à leur identification. *F oxysporum*, *F solani* var *coeruleum* et *F roseum* var *arthrosporioides* ont des zymogrammes bien distincts ; les profils d'estérases de *F roseum* var *culmorum* et de *F roseum* var *graminearum* sont proches tandis que celui de *F roseum* var *gibbosum* forme compactum est plus apparenté à celui de *F roseum* var *sambucinum* qu'aux 2 variétés précédentes. Les résultats obtenus concordent avec les subdivisions définies à partir de la morphologie. En cas d'homogénéité morphologique entre isolats, les isoestérases sont plus stables au niveau de l'espèce ou de la variété que dans le cas des taxons les plus polymorphes. La situation de variation importante est illustrée chez *F roseum* var *graminearum* où les 2 types d'isolats sont mis en évidence ; également chez *F roseum* var *arthrosporioides*, des modifications morphologiques observées s'accompagnent d'altérations isozymiques mais ces fluctuations n'éloignent pas ces variants de cette variété de *F roseum*. À ce sujet, la relation entre *F roseum* var *arthrosporioides* et *F roseum* var *avenaceum* est discutée. Enfin, cette méthode permet de mettre en évidence des différences entre souches de *F roseum* var *sambucinum* en fonction de leur sensibilité ou résistance au thiabendazole et de leur développement sur milieu de culture.

électrophorèse / isoenzymes / systématique / morphologie

INTRODUCTION

Fusarium is one of the most complex of the fungal genera. The current classification is based on morphological characteristics such as pigmentation of colonies on culture media, presence or absence of microconidia, shape of macroconidia, and presence or absence of chlamydospores. This multiplicity of criteria leads to determinative keys that differ according to the authors (Snyder and Toussoun, 1965; Messiaen and Cassini, 1981; Gerlach and Nirenberg, 1982).

Considering the degree of complexity encountered in the genus *Fusarium*, Booth (1971) suggested using biochemical criteria to better separate the species, varieties, and even *formae speciales*.

During the 1980s, the techniques for the electrophoresis of proteins and enzymes have proved useful for distinguishing fungal species, especially in genera where morphological criteria are difficult to apply, such as *Rhizopus* (Seviour *et al*, 1985), *Phytophthora* (Hansen *et al*, 1986; Bielenin *et al*, 1988), and *Peronosclerospora* (Micales *et al*, 1986).

Studies have been successfully carried out using esterases to discriminate between isolates or species of pathogenic agents, such as *Fusarium graminearum* (Drysdale and Bratt, 1971), *Rhizoctonia solani* (Matsuyama *et al*, 1978; Matsuyama, 1983), *Phytophthora* spp (De Vallavielle and Erselius, 1984), and *Ustilago* spp (Péros, 1985). Recently, this enzyme system alone was used by Angelstad and Stenersen (1991) in taxonomic studies of the genus *Eisenia*, by Nobbs *et al* (1992) to separate 4 species of the cyst nematode *Heterodera*, by Priestly *et al* (1992) to identify pathotypes of the eyespot pathogen *Pseudocercospora herpotricoides*. Using several enzyme systems some other workers have found esterase to be one of the most useful markers; Roig *et al* (1990) for comparing isolates of *Venturia inaequalis*, Somé and Samson (1991) for characterizing phytopathogenic fluorescent *Pseudomonas*, Simcox *et al* (1992) for determining races of *Cochliobolus carbonum*, Lehmann *et al* (1992) for the identification of the medically important yeast *Kluyveromyces marxianus var marxianus*.

Early work on the electrophoresis of *Fusarium* proteins did not result in a consensus as to the value of this method in terms of taxonomic differentiation within this genus. Meyer *et al* (1964), Hall (1967), Saito *et al* (1980), and Matsuyama

(1983) have successfully distinguished species and *formae speciales* of *Fusarium*. On the other hand, Glynn and Reid (1969) using only the soluble proteins as markers, do not follow the mainstream of opinion that this method could be used as a taxonomic tool.

The works of Reddy and Stahmann (1972) point to the importance of the choice of markers within the *Fusarium* genus. They used 13 enzyme systems to compare 5 *Fusarium* species and 6 special forms of *F oxysporum*, and found that 5 of these enzyme systems (either alone or in combination) could be used as taxonomic criteria.

In Brittany, several species or varieties of *Fusarium* have been isolated from potato tubers showing dry rot. In order to describe and facilitate detection of these fungi, Tivoli (1988) established an identification guide based on cultural and morphological characters. The present study compares this key with one generated using esterase isozymes as the analytic criteria.

MATERIAL AND METHODS

Strains

The strains of *Fusarium* used in this study came from the Station de Pathologie Végétale, Institut National de la Recherche Agronomique (INRA), Le Rheu, France and were isolated from batches of rotting potatoes produced in Brittany. From these, *Fusarium oxysporum*, *F solani var coeruleum*, and *F roseum* varieties *sambucinum*, *arthrosporioides*, *culmorum*, *graminearum*, and *gibbosum* form *compactum* (according to the classification of Messiaen and Cassini, 1968, 1981) were isolated. Correspondence with basic taxonomy and nomenclature of Wollenweber and Reinking (1935) was made (table I) from the illustrated manual for identification of *Fusarium* species of Nelson *et al* (1983).

The strains of each *Fusarium* species or variety isolated either from a different variety of potato or from a different locality were compared by isolating single conidiospores. Three single spore cultures were recovered for each strains to study intra-isolate variability.

Four isolates of *F roseum var graminearum* and 1 isolate of *F roseum var avenaceum* taken from wheat were provided by L Saur from the Station de Génétique et d'Amélioration des Plantes, INRA-Le Rheu. Altogether, 49 single-spore isolates (table II) were studied.

Culture methods

Six explants 4 mm in diameter from young malt (Merck, 5391) agar cultures were transferred asepti-

Table 1. Synonymy between the taxonomic systems of Messiaen and Cassini and Wollenweber and Reinking.

<i>Taxonomic system of Messiaen and Cassini (1981)</i>	<i>Sections</i>	<i>Taxonomic system of Wollenweber and Reinking (1935)</i> <i>Species</i>
<i>F solani</i> (Mart) Sacc var <i>coeruleum</i> (Sacc) booth	<i>Martiella</i> and <i>Ventricosum</i>	<i>F solani</i> (Mart) Appel and Wollenw emend Snyder and Hans <i>F coeruleum</i> (Libert) Sacc ^a
<i>F oxysporum</i> Schlecht emend Snyder and Hans	<i>Elegans</i>	<i>F oxysporum</i> Schlecht emend Snyder and Hans
<i>F roseum</i> LK emend Snyder and Hans var <i>sambucinum</i> (Fuckel) Snyder and Hans	<i>Discolor</i>	<i>F sambucinum</i> Fuckel <i>F sulphureum</i> Schlecht ^a
<i>F roseum</i> LK emend Snyder and Hans var <i>arthrosporioides</i> (Sherb) Messian and Cassini	<i>Sporotrichiella</i>	<i>F chlamydosporum</i> Wollenw and Reinking <i>F sporotrichioides</i> Sherb var <i>chlamydosporum</i> (Wollenw and Reinking)
<i>F roseum</i> LK emend Snyder and Hans var <i>avenaceum</i> (Sacc) Snyder and Hans	<i>Roseum</i>	<i>F avenaceum</i> (Fr) Sacc
<i>F roseum</i> LK emend Snyder and Hans var <i>graminearum</i> (Schwabe) Snyder and Hans – cereal type – soil type	<i>Discolor</i>	<i>F graminearum</i> Schwabe <i>F crookwellense</i> ^b Burgess, Nelson and Toussoun
<i>F roseum</i> LK emend Snyder and Hans var <i>culmorum</i> (Schwabe) (sic) Snyder and Hans	<i>Discolor</i>	<i>F culmorum</i> (WG Smith) Sacc
<i>F roseum</i> LK emend Snyder and Hans var <i>gibbosum</i> (Wollenw) Messiaen and Cassini <i>F compactum</i> (Tivoli)	<i>Gibbosum</i>	<i>F equiseti</i> (Corda) Sacc <i>pro parte</i> <i>F scirpi</i> Lambotte and Fautr var <i>compactum</i> Wollenw ^a

^a Nomenclature used by some authors in the case of the potato *Fusarium*; ^b this is a new species described by Nelson et al (1983).

Table II. List and origin of isolates of *Fusarium* studied according to the classification of Messiaen and Cassini, 1981.

Species or varieties of <i>Fusarium</i>	No of isolate	Host	Year of isolation	Origin	Remarks	Aggressiveness on potato tubers	Type of variability studied		
							Inter isolate	Intra isolate	
<i>F. roseum</i> var <i>culmorum</i>	FT1	Potato	1987	INRA-Le Rheu PP ^b		+	+		
	FT2	"	1987	"		+	+	+	
<i>F. roseum</i> var <i>graminearum</i>	FT3	"	1987	INRA-Le Rheu PP ^b	Soil type	+	+		
	FT4	"	1987	"	"	+	+		
	FT5	"	1987	"	"	+	+	+	
	FT6	"	1987	"	"	+	+		
	83-7	Wheat	1983	INRA-Le Rheu PG ^c	Cereal type	+	+		
	84-10	"	1984	"	"	+	+		
<i>F. roseum</i> var <i>gibbosum</i>	87-6	"	1987	"	"	+	+		
	87-7	"	1987	"	"	+	+		
	FT7	Potato	1987	INRA-Le Rheu PP ^b		0	+	+	
	FT8	"	"	"		0	+	+	
	FT9	Potato	1987	INRA-Le Rheu PP ^b		+	+		
	FT10	"	1987	"		+	+		
	FT11	"	1987	"		+	+		
<i>F. roseum</i> var <i>arthrosporioides</i>	FT12	"	1987	"		+	+		
	FT13	"	1987	"		+	+		
	83-19	Wheat	1983	INRA-Versailles PP ^b		+	+		
	FT14	Potato	1986	INRA-Le Rheu PP ^b	Sensitive to TBZ ^a	+	+	+	
	FT15	"	1986	"	"	+	+		
	FT16	"	1987	"	"	+	+		
<i>F. roseum</i> var <i>avenaceum</i>	FT17	"	1984	"	"	+	+		
	FT18	"	1987	"	"	+	+		
	FT19	"	1987	"	Tolerant to TBZ ^a	+	+		
	FT20	"	1987	"	"	+	+		
	FT21	"	1987	"	"	+	+		
	FT22	"	1987	"	"	+	+		
	<i>F. solani</i> var <i>coeruleum</i>	FT23	Potato	1987	INRA-Le Rheu PP ^b		+	+	
		FT24	"	1987	"		+	+	+
		FT25	"	1987	"		+	+	
	<i>F. oxysporum</i>	FT26	Potato	1987	INRA-Le Rheu PP ^b		0	+	+
FT27		"	1987	"		0	+		

^a Thiabendazole ; ^b Station de Pathologie Végétale ; ^c Station de Génétique et d'Amélioration des Plantes.

cally into a Roux flask containing 100 ml of the following liquid medium: 10 g glucose, 3.5 g KNO₃, 1.75 g KH₂PO₄, and 0.75 g Mg SO₄·7H₂O per 1 000 ml H₂O. This simple medium used by Bihari (1977) to culture *Collectotrichum capsici* is also suitable for the growth of *Fusarium*. The cultures were incubated at 20–24°C under 10 000 lux white light and a 12-h photoperiod. After 7 d, the culture was filtered through Whatman no 1 filter paper *in vacuo* to recover the mycelium, rinsed with distilled water, and then lyophilized. The mycelium could then be ground for immediate extraction or stored at –20°C for later use.

Protein extraction*

The method used was adapted from Seviour and Codner (1976): 50 mg Fontainebleau sand and 50 mg insoluble polyvinylpyrrolidone were added to 50 mg lyophilized mycelium in 1 ml extraction buffer kept at 4°C. This mixture was ground in a mortar (chilled to –20°C) for 15 min. The extraction buffer was adjusted to pH 7 with 1 N HCl containing 10 mM Tris, 1 mM EDTA, 3 mM 2-mercaptoethanol, and 1 mM cysteine. The supernatant was then centrifuged (at 2°C) at 13 000 g for 10 min to remove insoluble material, after which glycerol was added to a total of 10% of the final volume. This protein-rich solution was analyzed immediately or stored at –20°C.

Electrophoresis

One-way vertical electrophoresis on 0.75-mm thick by 70 x 85 mm 9% polyacrylamide gel was carried out according to Laemmli (1970) in a non-dissociating discontinuous system at pH 8.8 with 20 µl of extract solution in each well. Bromophenol blue dissolved in extraction buffer containing 10% glycerol was deposited in the outer wells to monitor migration. Electrophoresis was carried out in a Bio-Block Mini-Slab apparatus at 150 volts at 4°C until the migration front reached the bottom of the gel.

Esterase detection

Esterases were detected using a modified version of the method of Siciliano and Shaw (1976). 50 mg of Fast Blue RR salts were dissolved in 50 ml of 0.1 M phosphate buffer (pH 6) at 37°C. After filtration through Whatman no 1 filter paper, 20 mg α-naphthyl acetate and 20 mg β-naphthyl acetate dissolved in 1 ml acetone were added. After a few seconds of agitation, the gel was incubated in this mixture for 30 min at 37°C in the dark, then rinsed with water and fixed with 10% acetic acid.

Profile analysis

The criteria used for assessing the electrophoresis profiles were:

– the relative mobility (R_f) of the enzyme bands, according to Gottlieb and Hepben (1966):

$$R_f = bc/bf$$

where bc = migration distance of a given band; and

bf = migration distance of the leading band; and

– the band intensity, *ie* how intensely the isoesterase bands take up the staining material.

RESULTS

General observations

In the presence of α- and β-naphthyl acetates as substrates, esterase isozymes stain in 1 of 3 ways:

- a greenish color, indicating reaction with the α-substrate;
- a pinkish color, indicating reaction with the β-substrate;
- an intermediate color between these 2, resulting from simultaneous reaction of the enzyme with both substrates.

Six to 15 enzyme bands were seen for each isolate, depending on the species or variety. Slight intra- or inter-isolate variations did not alter the characteristic profiles of the various *Fusarium* cultures (figs 1, 2).

The different varieties of *F roseum* may be distinguished from *F solani* var *coeruleum* and *Fusarium oxysporum* by the presence of a greater number of isoesterases, and the presence of some identically stained enzyme bands that were common to some of them. These similarities include the isoesterases at R_f 0.29 in *F roseum* var *culmorum*, 0.33 in *F roseum* var *graminearum*, and 0.27 in *F roseum* var *arthrosporioides*; and the essentially β-isoesterases of R_f 0.44 in *F roseum* varieties *culmorum*, *graminearum*, and *sambucinum*, and 0.41 in *F roseum* var *gibbosum* form *compactum*. In addition, the enzyme profiles of *F roseum* var *culmorum* and *F roseum* var *graminearum* are almost identical, as are those of *F roseum* var *gibbosum* form *compactum* and *F roseum* var *sambucinum*.

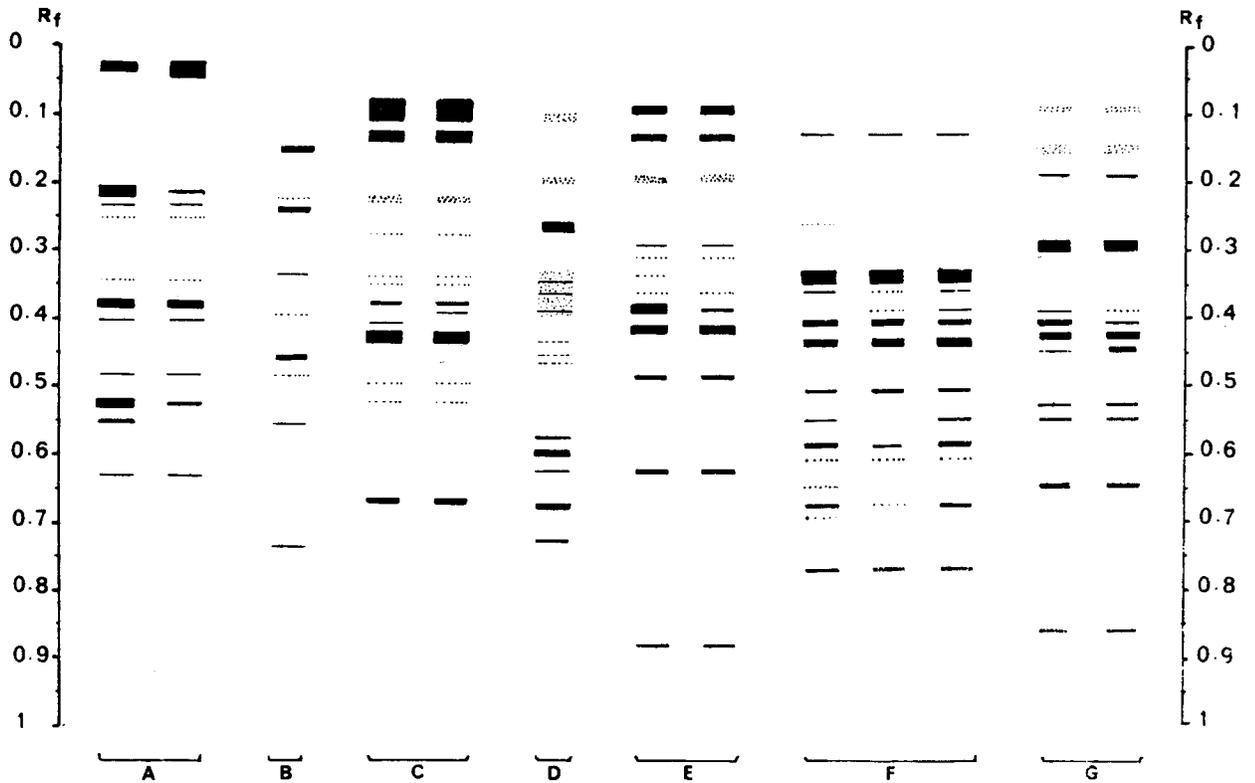


Fig 1. Variability of esterase patterns within a single isolate of each species or variety of *Fusarium*: *F. oxysporum* (A), *F. solani* var *coeruleum* (B), *F. roseum* var *sambucinum* (C), *F. roseum* var *arthrosporioides* (D), *F. roseum* var *gibbosum* form *compactum* (E), *F. roseum* var *graminearum* (F), *F. roseum* var *culmorum* (G).

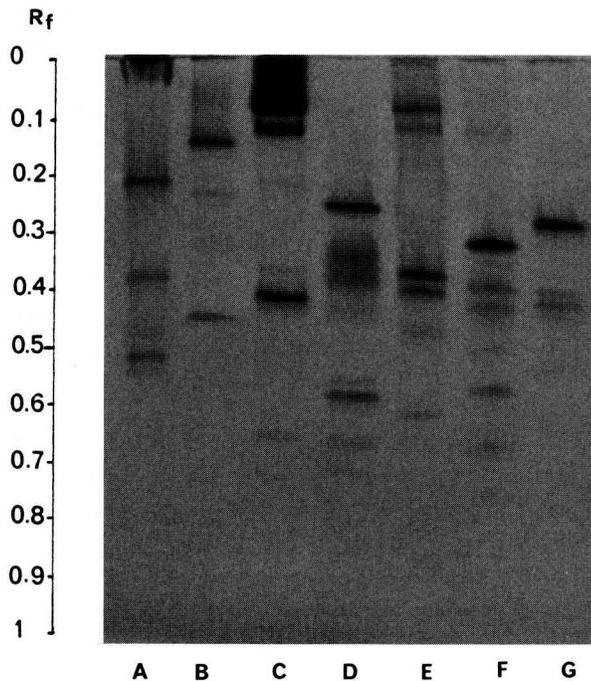


Fig 2. Esterase patterns of different *Fusaria* from potato: *F. oxysporum* (A), *F. solani* var *coeruleum* (B), *F. roseum* var *sambucinum* (C), *F. roseum* var *arthrosporioides* (D), *F. roseum* var *gibbosum* form *compactum* (E), *F. roseum* var *graminearum* (F), *F. roseum* var *culmorum* (G).

Observations by strain

Intra-isolate variability (fig 1)

This variability was evaluated by comparing the enzyme profiles of the 3 single-spore cultures from the same isolate for each species or variety of *Fusarium*.

Generally, the profiles of the 3 single-spore cultures from each species or variety of *Fusarium* had the same number of major bands. Single-spore cultures of *F. solani* var *coeruleum*, *F. roseum* var *arthrosporioides* showed the same profiles, while for *F. oxysporum*, *F. roseum* var *culmorum*, *F. roseum* var *sambucinum*, *F. roseum* var *gibbosum* form *compactum* and *F. roseum* var *graminearum*, variation between the 3 single-spore cultures concerned absence/presence or modification of the intensity of 1 to 3 minor bands.

Inter-isolate variability

No differences were detected in esterase profiles between isolates of *F. roseum* var *sambucinum*

and *F roseum* var *culmorum*. In *F solani* var *coeruleum*, the only variability among the 4 isolates examined concerned the intensity of the band at R_f 0.23.

F oxysporum

Each of the 2 isolates studied had a different profile: the first had 10 isoesterases, and the second had 6, 5 of which were in common to the first. Two (R_f 0.38 and 0.52) of the 4 major isoesterases were among the missing bands in the second isolate.

F roseum* var *sambucinum

Electrophoresis was carried out on 9 strains. Tivoli *et al* (1986) have shown that some strains of this variety are resistant to thiabendazole in malt agar medium (100 times the dose needed, *ie* 1 ppm, to inhibit growth of susceptible strains). Chosen strains covered both categories. Twelve isozymes were detected, 3 of which were major bands, but there was considerable variability between strains. Isoesterases at R_f 0.36, 0.64, and 0.67 (fig 3) could be present or absent, while those at R_f 0.08, 0.12, 0.21, 0.38, and 0.39 varied in intensity.

Using these differences in the enzyme profiles, we divided the strains into 4 types:

– Type I: presence of bands at R_f 0.36 and 0.64 (in strains FT14, FT15, FT16, and FT17);

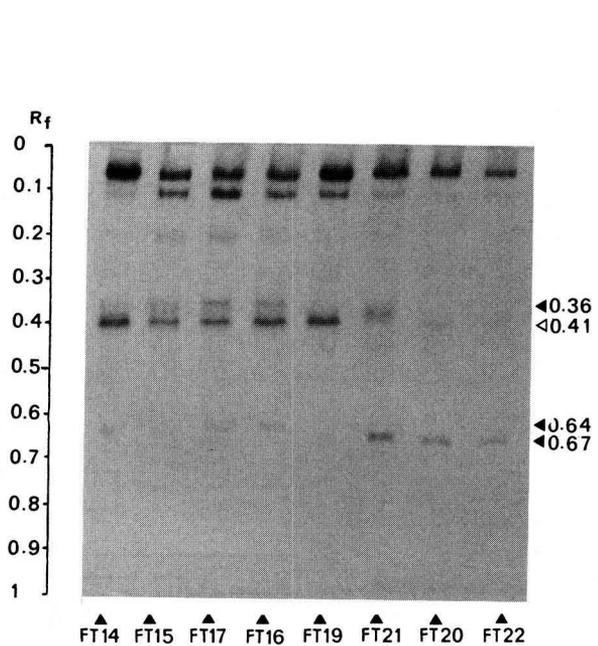


Fig 3. Esterase patterns of strains of *F roseum* var *sambucinum*.

– Type II: absence of isoenzyme at 0.36 and presence of one band at 0.67 (in strains FT20, FT21, and DT22);

– Type III: presence of the 2 bands at R_f 0.36 and 0.64 but the latter was particularly intense, plus the presence of the 2 other bands at R_f 0.69 and 0.71 (in strain FT18);

– Type IV: absence of the band at R_f 0.36 and presence at 0.67 but at low intensity (in strain FT19).

The thiabendazole-sensitive strains (Types I and III) possess the R_f 0.36 and 0.64 bands but lack the R_f 0.67 band, while the resistant strains (Types II and IV) are just the reverse.

F roseum* var *graminearum

Inter-isolate variability among the 4 isolates studied (fig 4a) concerned the intensity of the strongest band, and the presence, absence, and/or intensity of the 3 fastest migrating bands.

Observations on the varieties of *F roseum*

F roseum* var *graminearum

Isolates of this variety taken from the potato may be distinguished morphologically from those originated from wheat by the soil type shape (arched) or their macroconidia, while the

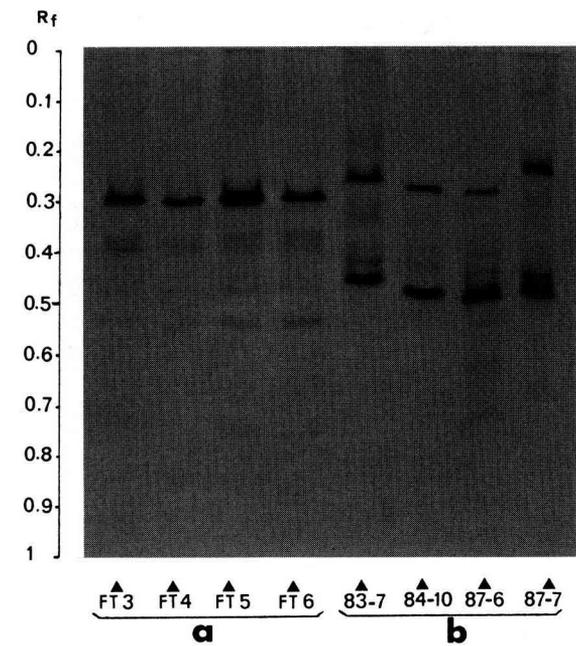


Fig 4. Esterase patterns of *F roseum* var *graminearum*: from potato (a), isolates from wheat (b).

cereal type shape is narrower and hardly curved (Messiaen *et al*, 1976).

To determine whether the electrophoretic data can be used to partition these isolates into different groups, 4 isolates from potato were compared with 4 isolates from wheat. The zymograms in figure 4 show an overall difference between the potato isolates (a) and the wheat isolates (b). In all, 30 different bands were detected. Figure 5 shows the number of bands that are different, comparing the zymograms two-by-two. The dendrogram in figure 6 is derived from figure 5 *via* simple relationships between the isolates, giving 2 groups. The first one, from potato, has few outliers and many bands in common, while the reverse is true for the second group isolated from wheat. However, isolate 87-7 is markedly different from the 2 groups.

F roseum var *arthrosporioides*

While the daughter cultures of single-spore isolates from the other *Fusarium* species and varieties were morphologically homogeneous, this situation did not always occur with *F roseum* var *arthrosporioides*:

– in the most frequent case (FT9, FT12, and FT13), the daughter single-spore isolates were morphologically identical to the parent cultures: that is, they were of the common type described by Tivoli (1988) with a deep carmine red color on contact with the culture medium. A representative zymogram is profile 2 in figure 7; these strains varied by the presence or absence of the

isozymes at R_f 0.65 and 0.75, and in the intensity of the bands at R_f 0.10, 0.26, 0.36 and 0.39;

– in the case of FT10, the daughter single-spore isolates were pink and morphologically identical, but different from the parent cultures. These single-spore cultures are an intermediate between the common carmine red type and the semi-albino type that has lost nearly all of its pigmentation. A zymogram of this isolate is profile 1 in figure 7, and is marked by the absence of bands at R_f 0.13, 0.19, 0.26, 0.36 and 0.39, and the exclusive presence of bands at R_f 0.29, 0.51, and 0.63;

– in the case of FT11, where the 4 daughter single-spore isolates were morphologically different both from each other and from the parent cultures (fig 8) the following was observed: 1 common type daughter isolate; 2 semi-albino type daughter isolates; 1 pionnotal type daughter isolate, characterized by a total lack of aerial mycelium, heavy production of *avenaceum*-type macroconidia, and an orange-red color. Their respective zymograms are profiles 2, 3, 3', and 4 in figure 7.

Profile 4 is identical to the esterase zymogram (profile 6 in figure 7) of an isolate of *F roseum* var *avenaceum* taken from wheat that also has the pionnotal-type morphology.

Approximately 20 enzyme bands were detected among the isolates of *F roseum* var *arthrosporioides* studied. The profiles thus obtained correspond better to the morphological characteristics than to the host origin of the isolates. Profiles 2, 3 and 4 (fig 7) correspond to an analysis of the intra-isolate variability since they are all derived from single-spore cultures of the same original isolate. In the zymograms, the intensity of the bands at R_f 0.34, 0.36, and 0.39 decreases among the semi-albino types and increases in the pionnotal type, relative to the common type, while the isoenzyme at R_f 0.26 which is strong in the common type, decreases in both of the other 2 types.

Thus, there is an intra-isolate variability that does not affect the number of enzyme bands, but may alter the intensity of some. Profiles 4 and 6 (fig 7), the 2 pionnotal types from *F roseum* va-

FT4	FT3	FT5	FT6	87-7	83-7	87-6	84-10	
0	2	1	4	13	18	17	15	FT4
	0	3	4	13	18	17	17	FT3
		0	3	14	19	18	18	FT5
			0	15	22	21	19	FT6
				0	11	16	14	87-7
					0	9	5	83-7
						0	6	87-6
							0	84-10

Fig 5. Number of different esterase isozyme bands in each isolate pair of *F roseum* var *graminearum*.

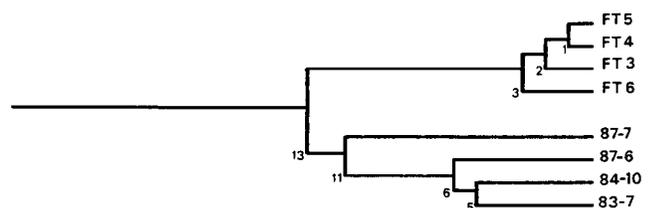


Fig 6. Clustering of the isolates of *F roseum* var *graminearum* by the minimum number of different esterase isozymes in an isolate pair (based on data from figure 5).

rieties *arthrosporioides* and *avenaceum*, respectively, show the same intensity for the bands at R_f 0.26, 0.34, 0.36, and 0.39.

Based on these observations, we may conclude that intra-isolate variability is greatest among these *Fusarium* taxa, both in esterase patterns and in morphology.

DISCUSSION

Congruence of morphological and electrophoretic grouping

The present study demonstrates the congruence between the morphological and electrophoretic

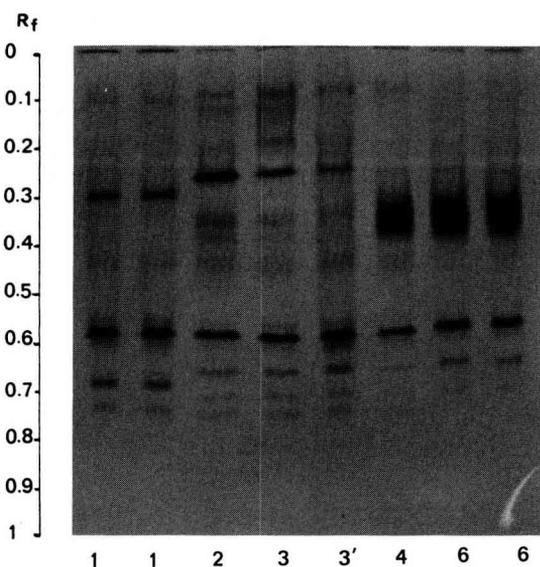


Fig 7. Esterase patterns of isolates of *F. roseum* var *arthrosporioides* belonging to different morphological groups: (1) isolate FT10, (2, 3, 3', 4) 4 single-spore isolates obtained from the isolate FT11 (see table I) and 1 isolate (83-19) of *F. roseum* var *avenaceum* (6).

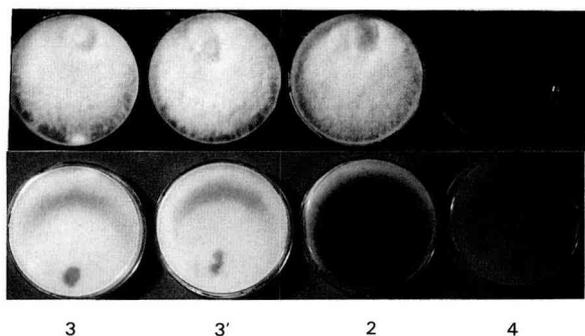


Fig 8. Morphology of 4 single-spore isolates on Tanaka medium (identification medium, Tivoli 1988) obtained from the same isolate of *F. roseum* var *arthrosporioides*, upper (top), lower (bottom). 2: common type; 3 and 3': 2 isolates belonging to the semi-albino type; 4: pionnotal type.

characters for the identification of *Fusarium* spp and varieties found in potato.

The esterases are a good example of an enzyme system that is highly discriminant for species as well as varieties of *Fusarium*. Assuming that the enzymes detected are coded by different alleles at different genetic loci, then we could conclude that the differences between the profiles reside in the fungal genome. In this case, the groupings detected via the zymograms are very similar to the species or variety subdivisions for *Fusarium* established by morphology.

The preliminary results from this study are essentially limited to the *Fusarium* spp found on potato, but it seems likely that this analysis could be extended to isolates of these *Fusarium* species and varieties that originated from different host plants. This extension would be of particular value in determining the importance of the host-source-dependent morphological variation observed in some varieties by Messiaen and Cassini (1968).

Analysis of variability at the species or variety level shows that there are slight variations in the esterase composition within each group. Nevertheless, strength and constancy of most enzyme bands can serve to group strains of *Fusarium* with major discriminative bands indicated in figure 9.

Relationships between F. roseum varieties

Our studies of *F. roseum* var *graminearum* showed that the potato isolates and wheat isolates belonged to 2 distinct groups and that there may be additional subgroups within the wheat group. Some isoesterases accentuate this separation: the potato isolates have a major band at R_f 0.31, with an affinity for the β -naphthyl acetate substrate. The wheat isolates have 2 major bands; one at R_f 0.22, 0.26 or 0.28 that are similar in staining to the band at R_f 0.31; and the other at R_f 0.49 or 0.51 that reacts exclusively with the α -naphthyl acetate substrate. These results based on biochemical criteria confirm the existence of at least 2 groups of isolates in *F. roseum* var *graminearum*, as proposed by Messiaen *et al* (1976) based on the morphology of their spores and host source. The potato isolates could probably belong to *F. crookwellense*, as described by Burgess *et al* (1982). These authors used one isolate from France illustrated by

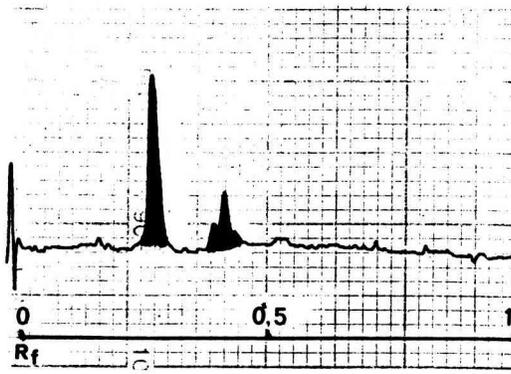
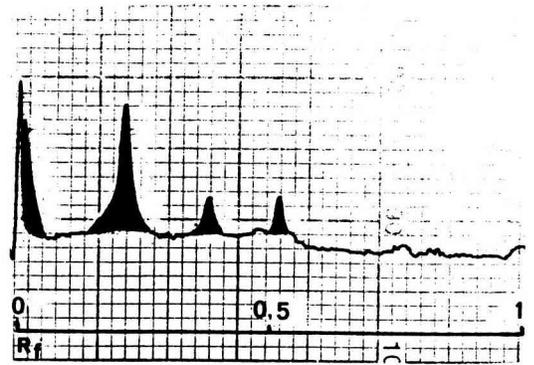
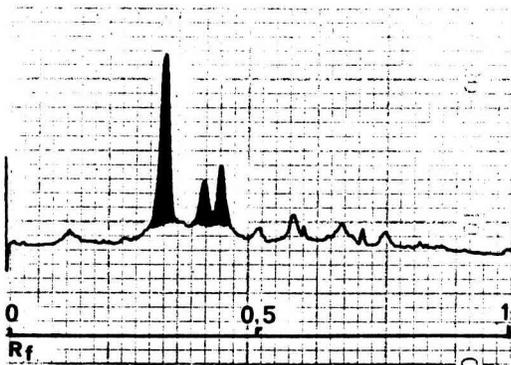
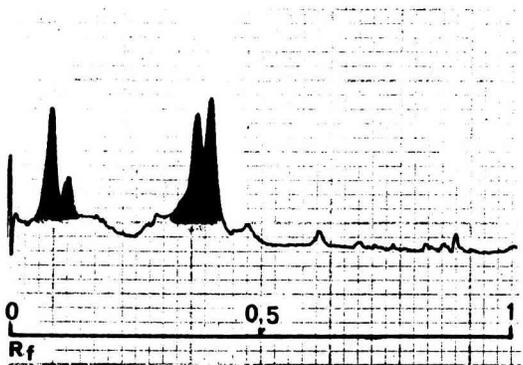
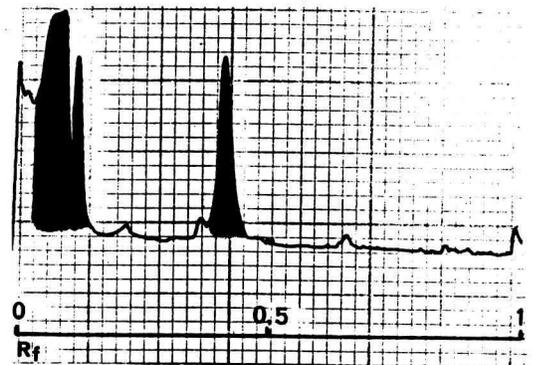
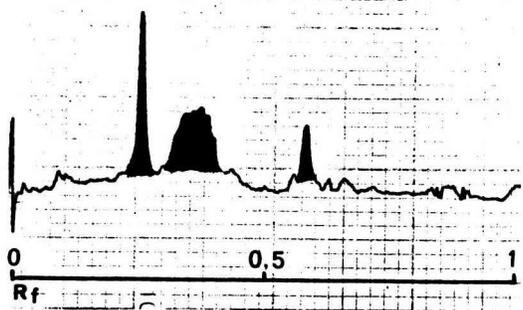
*F. roseum* var. *culmorum**F. oxysporum**F. roseum* var. *graminearum**F. solani* var. *coeruleum**F. roseum* var. *gibbosum* forme *compactum**F. roseum* var. *sambucinum**F. roseum* var. *arthrosporioides*

Fig 9. Densitometer scans of major isoesterases of each species or variety of *Fusarium* that could be used for identification.

Messiaen *et al* (1976) and consider that it is identical to *F crookwellense*.

The profiles were particularly useful in keeping the various morphological forms of *F roseum* var *arthrosporioides* together. On culture medium, the semi-albino variants of this group can easily be confused with *F oxysporum*, but their enzyme profile is that of *F roseum* var *arthrosporioides*. Based on Tivoli's (1988) determination criteria, the pionnotal type might not be classified as *F roseum* var *arthrosporioides*: however, the esterase profile of this strain is consistent with its assignment to this variety. In addition to showing this commonality, the refinement of this enzymatic system brings to light variations in intensity of certain isoesterases which correlate with morphological variations.

The limit between *F roseum* var *arthrosporioides* and *F roseum* var *avenaceum* has been difficult to detect. By studying the morphology and pathogenicity of several isolates of both varieties from different plant hosts, Cormack (1959) concluded that the criteria distinguishing these varieties were not sufficiently precise, while Tivoli (1988), also using morphological criteria concluded that *F roseum* var *arthrosporioides* and *F roseum* var *avenaceum* from potato are the same variety of *roseum*.

Although the number of samples is limited, we found that *F roseum* var *avenaceum* possesses the constant and characteristic bands of *F roseum* var *arthrosporioides*. Additionally, the bands at R_f 0.34, 0.36, and 0.39 were the same for the pionnotal forms of the both varieties. This similarity in their zymograms is supported by their identical morphology and the low aggressiveness of the 2 variants. These 2 varieties could be clonal, and more sensitive techniques will be required to distinguish them.

These pionnotal variants could be considered as degenerate forms of *F roseum* var *arthrosporioides*. In addition to enzymatic modifications relative to the common type, a reduction in growth was observed on liquid culture medium, as well as a decrease in aggressiveness, although the symptoms observed in the potato tuber are qualitatively identical to those of the common type. These variants appeared only following single-spore isolation, suggesting that they are at a competitive disadvantage relative to the common type under field conditions. This conclusion is consistent with the proposal of Cormack (1959) who observed that the pionnotal variants had a relatively short lifespan in soil culture, which

could explain their relative rarity in soil and other natural environments.

Thiabendazole-resistance in *F roseum* var *sambucinum* and its esterases

Four enzyme profiles were found for *F roseum* var *sambucinum*. These profiles are correlated with the strain's response to thiabendazole, and their development on Bihari's (synthetic) medium. In general, sensitive strains develop well (Type I) on this medium, while resistant strains develop more slowly (Type II). These 2 groups correspond to those already noted by Langerfeld (1986) in *F sulphureum*. This investigator also observed that thiabendazole-sensitive strains had a normal growth on PDA and Czapek media, while resistant strains were strongly inhibited on the latter medium. Among the strains we analyzed, we noted 2 new groups: Type III (both thiabendazole-sensitive and Bihari's growth inhibited), and Type IV (thiabendazole-resistant with normal growth on Bihari's medium). It is possible that these 2 newly identified types are intermediates between the 2 previously identified types which are more common. On the other hand, our data indicate a difference in nutritional requirements between strains that is independent of fungicide resistance, suggesting that fungicide resistance and nutritional requirements are distinct, even though in most cases they appear to be correlated.

CONCLUSION

Grouping strains of *Fusarium* from potato *via* esterase zymograms is consistent with the grouping based on morphological criteria.

The morphological variability (for example, in *F roseum* var *graminearum* and *F roseum* var *arthrosporioides*) is correlated with changes in esterase isozyme profiles. Conversely, the absence of morphological variation reflects a certain stability in the esterases at the level of the species or variety.

Since the esterases are easy to detect, this enzymatic system may be incorporated into a key for identifying different *Fusarium* species and subspecies. Before this step is taken, however, a greater number of isolates should be analyzed to define the limits of isozyme variability within each group, so that a characteristic

profile for each species or variety can be established.

Lastly, while the esterases allow various *Fusarium* taxa to be identified, they are not sufficient for determining familial relationships between them. To help clear up some of the taxonomic problems within the species *F. roseum*, it could be envisaged, as a complement to the esterases, to use other enzyme systems likely to present greater homology between the isolates of the different varieties, but also certain recent methods such as the technique of sequencing homologous portions of ribosomal RNA, used in the study of phylogeny (Guadet *et al*, 1989).

ACKNOWLEDGMENTS

We thank M Le Normand (director of the Chair of Botany and Plant Pathology at the École Nationale Supérieure Agronomique de Rennes (ENSAR)) for providing the electrophoretic materials, D Chicouène (Laboratory of Plant Ecology, University of Rennes I and Laboratory of Botany and Plant Pathology at ENSAR) and J Citharel (Director of the Laboratory of Plant Physiology, University of Rennes I) for helpful suggestions in the preparation of this manuscript, and J Marie (INRA-Versailles) for the translation from French into English.

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