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Phenotypic characterization of natural populations of *Fusarium oxysporum* in relation to genotypic characterization

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Abstract

Sixty strains of *Fusarium oxysporum* were characterized by (i) in vitro growth parameter estimates on four carbon sources and (ii) vegetative compatibility grouping. Growth was assessed by measuring optical density changes of fungal cultures in microtiter plates. Principal component analyses of growth parameter estimates permitted a good discrimination of the isolates on the four carbon sources and revealed a high level of diversity within populations of *F. oxysporum*. The 60 strains were assigned to 40 vegetative compatibility groups. Strains grouped in a given vegetative compatibility group had similar growth parameters. Trophic characterization and vegetative compatibility groups were compared to a genotypic characterization previously performed on this collection. Trophic characterization and vegetative compatibility grouping were more discriminating than the genotypic characterization. The three characterization methods suggested that vegetative compatibility groups could be considered the population unit among natural *F. oxysporum* populations.

Keywords: Fungal growth; Genotypic characterization; Growth parameter; Non-pathogenic *Fusarium oxysporum*; Soil-borne fungus; Vegetative compatibility group

1. Introduction

Fusarium oxysporum Schlect. emend. Snyd. & Hans. is a common soil-borne fungus that is ubiquitous in soil [1]. Some intraspecific populations are pathogenic and responsible for wilts or root rots while others appear to be non-pathogenic. Pathogenic strains cannot be distinguished from non-pathogenic ones except when inoculated into the host plant. However, both play an important ecological role. More than 120 pathogenic *formae speciales* and races have been described [2]. The non-pathogenic populations are able to limit disease incidence in naturally suppressive soils [3,4] and in conducive soils and soilless cultures when inoculated as biocontrol agents [5–7]. Several mechanisms have been put forward to explain the effectiveness of non-pathogenic *F. oxysporum* in suppressing diseases. Non-pathogenic strains of *F. oxysporum* are able to induce resistance to fusarium wilts in various plant species [8–11]. Non-pathogenic populations can efficiently compete with pathogenic populations in soil for nutrients [12,13], on roots for infection sites [7,14] and pene-

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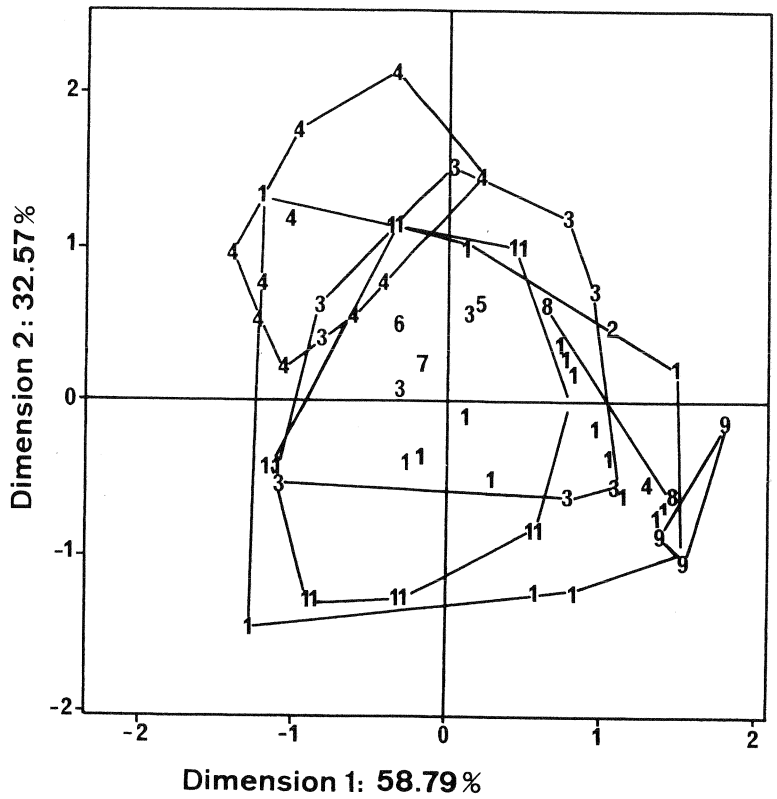

Fig. 1. Principal component analysis (PCA) performed on the growth parameters of each replicate for each strain of *F. oxysporum* on glucose. PCA was performed using the five replicates for each parameter but only the average was plotted on the figure. The strains are represented by the number of the IGS type to which they were assigned. The peripheral points of each cloud of strains belonging to the same IGS type are joined. Strains on the right of the figure had higher r and K values than strains on the left. Above the first component axis (horizontal axis) are strains with a longer lag phase than strains situated below this axis.

tration [15]. Competition between pathogenic and non-pathogenic populations can result in significant reductions of disease incidence. One may assume that opportunistic populations able to germinate rapidly, and exhibiting the highest growth rates, would have a great competitive advantage resulting in high population levels in the rhizosphere and should be effective antagonists. Growth parameters can greatly differ among *F. oxysporum* strains [16] and a positive correlation may exist between growth parameters, root colonization abilities and antagonistic efficacy [17]. Therefore, it could be of interest to divide large samples of *F. oxysporum* populations into subgroups on the basis of their growth parameters, but there is no efficient method to evaluate the trophic diversity of these populations. Conversely, intraspecific diversity within natural populations of *F. oxysporum* has been frequently assessed using vegetative compatibility groups (VCG) [18–20] and various molecular methods [21–23].

The aim of the present study was to establish a method, based on growth parameter evaluation, to characterize the diversity of natural populations of non-pathogenic *F. oxysporum* and to compare this phenotypic characterization with VCG and a genotypic characterization.

2. Materials and methods

2.1. Fungal strains

Strains of *F. oxysporum* were isolated either from a clay loam soil or from the roots (rhizoplane and

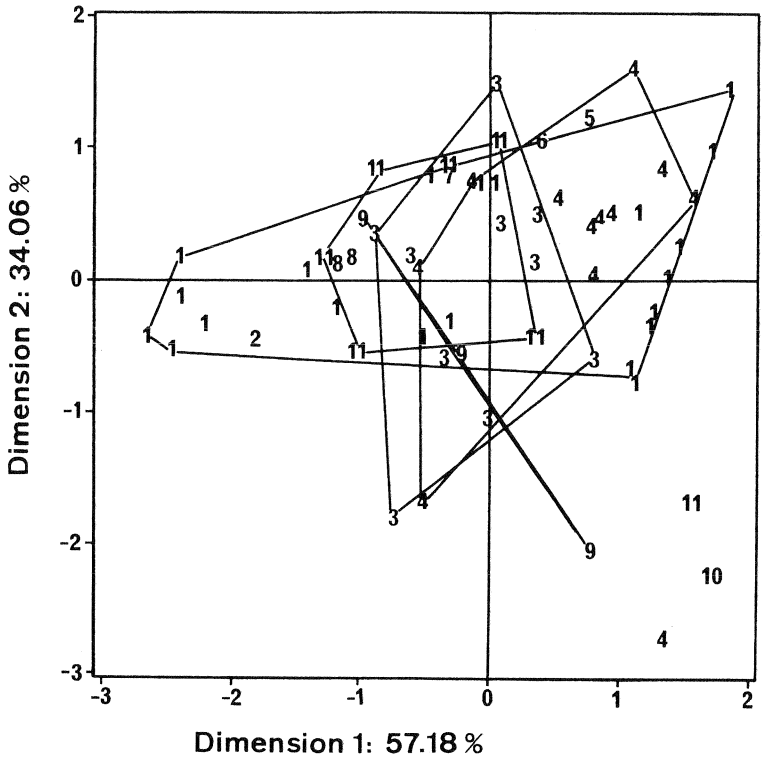

Fig. 2. Principal component analysis (PCA) performed on the growth parameters of each replicate for each strain of *F. oxysporum* on galactose. PCA was performed using the five replicates for each parameter but only the average was plotted on the figure. The strains are represented by the number of the IGS type to which they were assigned. The peripheral points of each cloud of strains belonging to the same IGS type are joined. The strains situated on the right of the figure had smaller r and K values than strains situated on the left. Above the first component axis (horizontal axis) are strains with a longer lag phase than strains situated below this axis.

root tissues) of four plants, flax (*Linum usitatissimum* L., cv. Opaline), tomato (*Lycopersicon esculentum* Mill., cv. H63.5), wheat (*Triticum vulgare* Vill., cv. Ventura) and melon (*Cucumis melo* L., cv. Tendral), grown in this soil. Isolation was performed using Komada's selective medium [24]. The strains were morphologically identified as *F. oxysporum* according to Nelson et al. [25]. Several hundred isolates were collected by single spore transfer and stored by cryopreservation at −80°C. A subsample of 60 strains was randomly selected. The 60 strains were previously characterized using a genotypic method described for the characterization of large populations of *F. oxysporum* [23]. This method was based on restriction fragment analysis of polymerase chain reaction (PCR)-amplified ribosomal intergenic spacer (IGS). Each isolate was assigned to an IGS type defined by the combination of the restriction patterns obtained with nine restriction enzymes. Eleven IGS types (IGS types 1–11) were recorded among the 60 strains.

2.2. Pathogenicity tests

The 60 strains of *F. oxysporum* were tested for their pathogenicity on flax, melon and tomato. Strains were cultivated in liquid malt extract (10 g l^{-1}, pH = 5.5) for 5 days on a rotary shaker (150 rpm) at 25°C. The whole culture was filtered on a sterile sintered glass funnel (maximum pore size 40–100 μm) and the suspension of conidia in the filtrate was adjusted to 9×10^5 conidia ml^{-1}. For each strain, three independent conidial suspensions originating from three independent precultures were prepared. The plants were grown in growth chambers with appropriate conditions for each plant species.

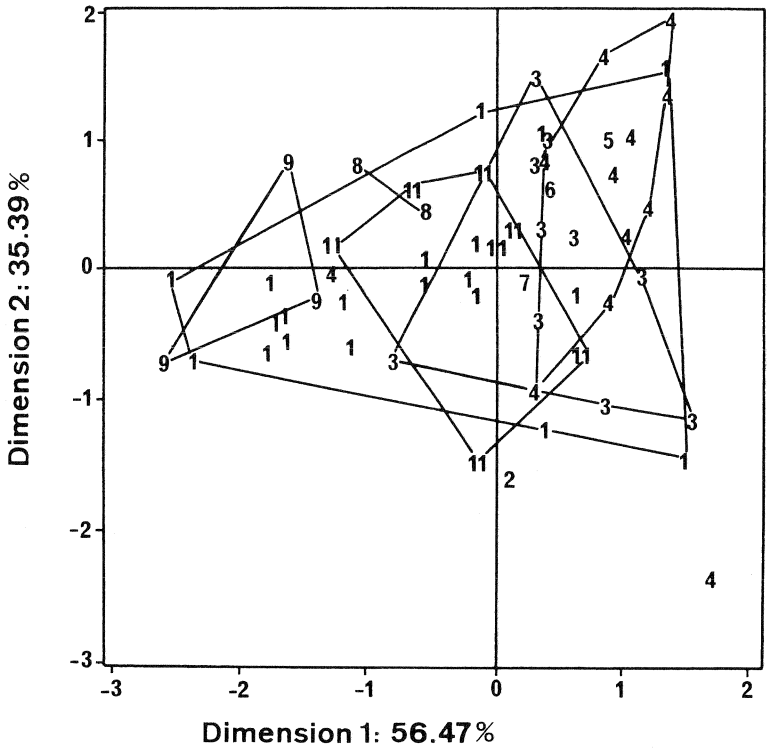

Fig. 3. Principal component analysis (PCA) performed on the growth parameters of each replicate for each strain of *F. oxysporum* on maltose. PCA was performed using the five replicates for each parameter but only the average was plotted on the figure. The strains are represented by the number of the IGS type to which they were assigned. The peripheral points of each cloud of strains belonging to the same IGS type are joined. The strains situated on the right of the figure had smaller r and K values than strains situated on the left. Above the first component axis (horizontal axis) are strains with a longer lag phase than strains situated below this axis.

Each plant was grown in a 9 ml rockwool plug wetted twice a day with nutrient solution. Two weeks after sowing, each plant was inoculated with 1 ml of the fungal suspension. Each fungal suspension was used to inoculate six replicate plants resulting in 18 inoculated plants per isolate. Controls consisted of plants inoculated with reference pathogenic strains of *F. oxysporum* of flax, melon and tomato (strain Foln3, Fom15 and Fol32 respectively, from the FPFS culture collection, INRA, Dijon, France). Typical symptoms of fusarium wilt appeared in the controls 3 weeks after inoculation with the pathogenic strains. They consisted first in a yellowishing of leaves which then turned completely to yellow, wilted and dried up. The plants were checked for symptoms once a week for 8 weeks after inoculation. At that time, almost all of the control plants were wilted while no symptoms were detected in the plants inoculated with the strains under test.

2.3. Trophic characterization

Trophic characterization was assessed by estimating the growth parameters of the 60 strains singly on four different carbon sources (glucose, maltose, sucrose and galactose) in the liquid minimal medium (LMM) described by Correll et al. [26], but without any agar. Trace element solution [26] was filter sterilized and added to the medium before use. Stock solutions of each of the C sources were prepared (0.4 g ml^{-1}), filter sterilized and an appropriate aliquot was added to LMM before use to provide a final concentration of 1.6 g C l^{-1}.

Each strain was precultivated in LMM supplemented with the C source for 5 days on a rotary shaker at 25°C. Mycelium mats were discarded by filtration. The conidial suspension was then adjusted to 4×10^3 conidia ml^{-1} and was used to inoculate five wells of a microtiter plate (50 μl per well),

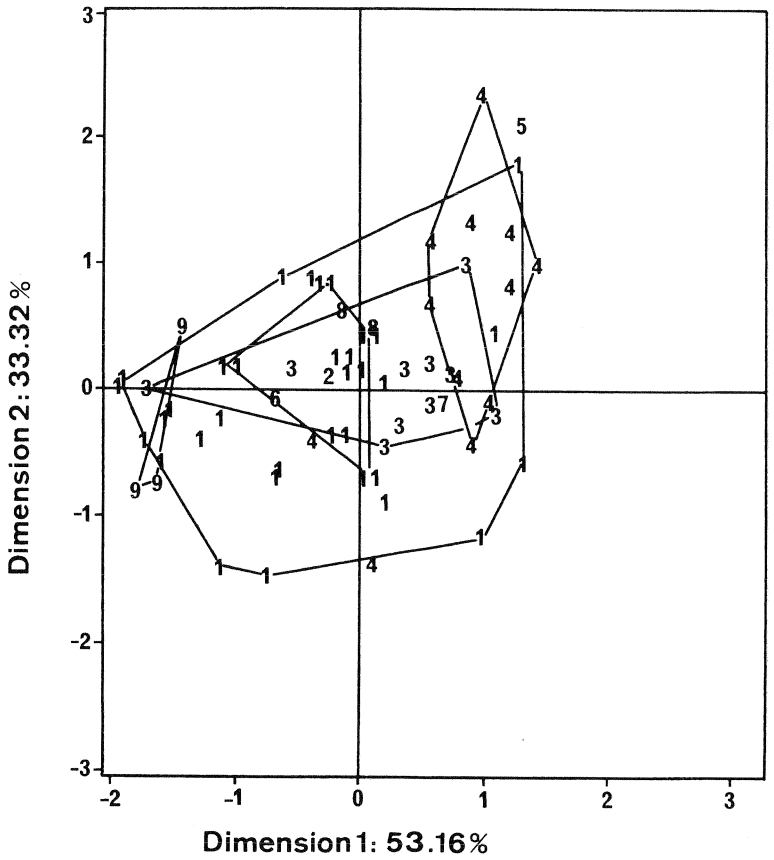

Fig. 4. Principal component analysis (PCA) performed on the growth parameters of each replicate for each strain of *F. oxysporum* on sucrose. PCA was performed using the five replicates for each parameter but only the average was plotted on the figure. The strains are represented by the number of the IGS type to which they were assigned. The peripheral points of each cloud of strains belonging to the same IGS type are joined. The strains situated on the right of the figure had smaller r and K values than strains situated on the left. Above the first component axis (horizontal axis) are strains with a longer lag phase than strains situated below this axis.

each containing 150 μl of LMM supplied with the same C source that was used for the preculture. One well containing 200 μl of LMM+C source was kept uninoculated for each set of five inoculated wells. This well was the reference optical blank for these five inoculated wells. Four strains on the four C sources (i.e., 16 different treatments) were plated per microtiter plate. The plate was incubated at 25°C, in the dark. Optical density (OD) measurements ($\lambda = 650$ nm) were performed for 120 h at

Table 1
Co-ordinates of the vectors of the parameters for each of the principal component analyses performed on the four C sources

	C source							
	Glucose		Maltose		Galactose		Sucrose	
	Dimension 1	Dimension 2	Dimension 1	Dimension 2	Dimension 1	Dimension 2	Dimension 1	Dimension 2
R (= 1/r)	−0.673	0.289	0.713	0.151	0.712	0.02	0.707	−0.001
P	0.237	0.956	−0.067	0.957	0.317	0.88	0.15	0.977
K	0.701	−0.046	−0.698	0.247	−0.626	0.47	−0.692	0.211

Table 2
Ribosomal intergenic spacer (IGS) types and vegetative compatibility groups (VCG) of 60 non-pathogenic strains of *F. oxysporum*

IGS type	VCG	Number of isolates
1	1	8
	2	4
	3	2
	4	2
	5	1
	6	1
	7	1
	8	1
2	9	1
3	10	2
	11	1
	12	1
	13	1
	14	1
	15	1
	16	1
	17	1
	18	1
4	19	3
	20	2
	21	1
	22	1
	23	1
	24	1
	25	1
	26	1
	27	1
5	28	1
6	29	1
7	30	1
8	31	1
	32	1
9	33	2
	34	1
10	35	1
11	36	2
	37	2
	38	1
	39	1
	40	1
	ND[a]	1 SI[b]

[a]Not determinable.
[b]Self-incompatible strain.

regular time intervals using a microtiter plate reader (Thermomax, Molecular Device®, USA). The lid of the microtiter plate was removed for each reading. The reader was monitored by an Apple MacIntosh® computer that automatically recorded the data.

First of all, it was necessary to ensure that the OD increases were correlated with fungal biomass increases. Because the dry weight biomass could not be assessed accurately by any means in the wells of microtiter plates, a two-step experiment was carried out. This two-step experiment consisted of establishing (i) a relation between dry weight biomass and protein content of a culture of *F. oxysporum* grown in flasks and (ii) a relation between OD measurements and protein content of a culture of *F. oxysporum* grown in microtiter plates.

(i) Dry weight and protein content measurements in flasks: 72 250 ml conical flasks containing each 100 ml of LMM+glucose were inoculated with 1 ml of a *F. oxysporum* conidial suspension adjusted to 10^6 conidia ml^{-1}. The flasks were incubated on a rotary shaker (150 rpm) at 25°C. At regular intervals, six flasks were randomly taken and their content was independently filtered on glass fiber filters (Whatman GF/A). Three filters were previously weighed. These filters plus the fresh biomass retained on them were placed in an oven at 105°C for 24 h to determine the dry weight biomass. The fresh biomass retained on the three other filters was carefully peeled off the filter, placed respectively into three mortars and crushed thoroughly with a pestle in the presence of liquid nitrogen. Each pellet was resuspended in 25 ml sterile distilled water. The suspension was filtered on Whatman glass fiber filters and the filtrates were frozen for subsequent protein content determination. This determination was carried out on the same day for all the samples. For each filtrate, three subsamples were prepared and the protein content was determined by Brad-

Table 3
Mean values of the growth parameters of the isolates belonging to the non-single-member VCGs when grown in LMM+glucose

VCG number	r (h^{-1})	P (h)	K (OD units)
27	0.325^{a}	$75.96^{c,d}$	1.73^{a}
1	0.249^{b}	$75.49^{c,d}$	1.47^{b}
2	$0.215^{b,c}$	$72.95^{c,d}$	1.68^{a}
36	$0.194^{b,c,d}$	$76.15^{c,d}$	1.27^{b}
3	$0.175^{c,d}$	78.26^{c}	1.43^{b}
4	0.156^{d}	$82.64^{a,b}$	1.39^{b}
10	$0.128^{d,e}$	$79.01^{b,c}$	1.31^{b}
19	0.095^{e}	$80.04^{b,c}$	1.01^{c}
20	0.092^{e}	84.96^{a}	0.91^{c}
35	0.086^{e}	$76.85^{c,d}$	1.34^{b}

Multiple comparisons of the means were achieved using Duncan's test. The same letter (a–e) indicates means which are not statistically different (significant level $P = 0.05$) within a column.

ford's micromethod [27] using the Biorad® kit for protein assay.

(ii) OD and protein content measurements in microtiter plates: the wells of a microtiter plate were filled with 150 μl LMM+glucose and 16 randomly distributed wells were filled with an extra 50 μl LMM+glucose to act as reference optical blanks. The other 80 wells were inoculated with 50 μl of a conidial suspension of a *F. oxysporum* strain as described above. Incubation conditions were similar to those described for the microplate assay. At regular time intervals, OD measurements were performed and the contents of five randomly chosen wells were removed and diluted in 5 ml distilled water in a test tube. The tube was then placed into ice and the sample was sonicated for 1 min at 50 W using a 3 mm diameter probe. The size of the sample imposes sonication although protein extraction is better achieved by crushing the sample in a mortar in the presence of liquid nitrogen than by sonication (data not shown). Samples were then centrifuged (5 min, $5000 \times g$) and the protein content of the supernatant was determined as before.

Water losses due to evaporation were estimated by regular weighings of a non-inoculated microplate where the wells were filled with 200 μl LMM and which was incubated under the same conditions. Border effects were checked by comparing growth kinetics of a *F. oxysporum* strain in border and inner wells.

Highly significant correlations were found (i) between dry weight biomass and protein content ($r = 0.958$, $P = 0.01$) and (ii) between OD measurements and protein content ($r = 0.944$, $P = 0.01$) indicating that OD increases corresponded to fungal biomass increases. Water losses were found to be low and constant (0.29 ± 0.06 μl h^{-1} $well^{-1}$) during the course of incubation. Growth kinetics of *F. oxysporum* strains were identical in border wells and in inner wells when growth lasted less than 120 h. Thus, growth kinetics were measured within a time that did not exceed 120 h.

These preliminary tests allowed the use of microtiter plates to estimate growth kinetics of *F. oxysporum* isolates by OD measurements.

2.4. Identification of vegetative compatibility groups

Vegetative compatibility was determined by pairing complementary nitrate non-utilizing (*nit*) mutants derived from each of the 60 strains examined, as previously described by Correll et al. [26]. Briefly *nit* mutants were generated on minimal medium (MM)+1.5% $KClO_3$+0.16% L-asparagine [28] and assigned to one of three classes (i.e., *nit*1, *nit*3 and NitM) based on their phenotype on media containing one of five different nitrogen sources (nitrate, nitrite, hypoxanthine, ammonium and uric acid). Pairings were made on minimal medium in 24-well tissue culture plates [29]. Drops of a conidial suspension of a *nit*1 and a NitM mutant from each strain were placed on opposite sides of wells in all possible combinations. Control wells were set up to check each mutant by itself for possible reversion. Genetic complementation was indicated by the presence of dense aerial growth where the mycelia of the two *nit* mutant colonies came into contact and formed a heterokaryon. A *nit*3 mutant was used either when no NitM was obtained or when the *nit*1 or the NitM mutant from a strain reverted to the wild-type phenotype. Control pairings between complementary mutants derived from the same parental isolate were made to detect any strain that might be heterokaryon self-incompatible [30]. All pairings were repeated at least three times.

2.5. Mathematical modelling and statistical analysis

Growth data were recorded well by well as de-

scribed above, providing five independent growth kinetic estimates per isolate and per C source. The tests provided four sets (one set for each C source) of 300 kinetic estimates each. The SAS procnlin [31] procedure was used to fit the logistic model to growth kinetics. This model was chosen to describe the evolution of fungal populations against time as experimental curves showed a typical sigmoid pattern which could be represented by:

$$dx/dt = K/1 + \exp((P-t)/R)$$

where x is population biomass (as measured by optical density), K is population density at the plateau, i.e., the carrying capacity of the medium for this isolate, P is the value of t when $x = K/2$ (provides information on the length of the lag phase), $1/R$ is r (growth rate of the strain) and t is time.

The three parameters (r, K, P) were estimated independently for each growth kinetic estimation resulting in five sets of three parameters per strain and per C source.

A principal component analysis (PCA) was then performed on the resulting estimated growth parameters. PCA was chosen since this analysis does not involve an a priori hypothesis on the structure of the data, contrary to discriminant analysis. In all cases, the PCAs were performed on the whole set of parameters estimated on a given C source (i.e., 3 growth parameters × 60 strains × 5 replicates per strain) but for the sake of clarity, only the average of the five replicates was used for the plotting procedure. Moreover, each strain is represented on the graph by its IGS type number as defined by the genotypic characterization. The peripheral points of each cloud of isolates belonging to the same IGS type were then joined by a line. The PCA programs and the plotting programs were written using the SAS language with references to Schlich's library [32].

One-way analyses of variance (ANOVA) were performed using the five replicates per isolate, to test for differences among the means of the growth parameters calculated for the non-single-member VCGs. Multiple comparison of the means was then achieved using Duncan's test [33]. First, homogeneity of all the means at a level γ_k is tested. If the result is a rejection, then each subset of $k-1$ means is tested at a level γ_{k-1}; otherwise the procedure stops.

3. Results

3.1. Pathogenicity tests

None of the 60 strains of *F. oxysporum* caused disease on flax, tomato or melon while reference strains of *F. oxysporum* f.sp. *lini* (Foln3), f.sp. *lycopersici* (Fol32) and f.sp. *melonis* (Fom15) induced wilt on flax, tomato and melon, respectively. Although potential pathogenicity on other host plants was not investigated, the 60 strains were considered non-pathogenic *F. oxysporum*.

3.2. Trophic characterization

3.2.1. Growth kinetics

The five growth curves measured in five wells for a given strain on a given C source were quite reproducible. The coefficient of variation within replicates was less than 5%. The general shape of the curves showed three phases: (i) a phase with no apparent growth (= lag phase) that lasted up to 30–45 h, depending on the strain and the C source; (ii) a phase with an exponential increase of the OD (exponential growth phase), (iii) and finally a decrease of the apparent growth rate while the curves reached a plateau (stationary phase). The sigmoid shape of the growth curves could be described by a logistic curve. The procnlin procedure of SAS [31] was successfully used to estimate r (specific growth rate), K (plateau value) and P (time value when x was equal to $K/2$) for each individual growth replicate.

A set of growth parameters was obtained for all the isolates on each of the four C sources. The values of the parameters varied according to the strain. On LMM+glucose, growth rates ranged from 0.058 h^{-1} to 0.353 h^{-1}, K values ranged from 0.48 OD unit to 1.79 OD unit and P values ranged from 56 h to 88 h. Similar ranges of variation were observed for estimates of growth parameters on the other C substrates.

3.2.2. Principal component analysis

A PCA was performed on each set of growth parameter estimates within each C source. Figs. 1–4 show the results of the PCAs performed on growth parameters estimated on glucose, galactose, maltose and sucrose, respectively. In all cases, two compo-

nents provided a good representation of the data, accounting for more than 80% of the standardized variance. In the case of glucose, as shown in Table 1, there was a negative correlation between the first principal component axis and R ($=1/r$) and a positive correlation between the first principal component axis and K. P was also strongly positively correlated with the second principal component axis. Therefore, strains could be classified according to their growth behavior on the C sources: strains on the right of the first principal component axis had both higher r and K values than strains on the left side. Above the first axis were strains with a high P value, i.e., with a longer lag phase, than strains below the first axis. Conversely, in the case of the three other C sources, K was negatively correlated with the first principal component axis while R was positively correlated, P remaining positively correlated with the second principal component axis. Therefore strains on the right part of the first axis (Figs. 2–4) have smaller r and K values than strains on the left part. Discrimination between groups according to their growth parameters was better when disaccharides (maltose and sucrose, Figs. 3 and 4 respectively) were used as a C source than when monosaccharides (glucose and galactose, Figs. 1 and 2 respectively) were used. Whatever the C source, isolates belonging to IGS type 1 and to a lesser extent IGS type 3 were always dispersed, exhibiting both high and low growth rate or K values, or both long and short lag phase values.

In the case of galactose (Fig. 2) none of the groups could be distinguished from the others while in the case of glucose (Fig. 1) and of the disaccharides (Figs. 3 and 4), strains from IGS type 4 on the one hand and strains from IGS type 9 on the other hand appeared different from the others. The former (IGS type 4) could be characterized by a lower growth rate, a lower K value and a higher lag phase than most strains from the other IGS types. In contrast, strains from IGS type 9 had higher r and K values than most other strains. However, only three isolates belonged to IGS type 9, thus limiting any extrapolation.

In none of the cases was P a good parameter to discriminate between the groups with the exception of IGS type 9 and IGS type 4 in the case of glucose. Strains belonging to IGS type 9 had a shorter lag phase than strains from IGS type 4.

3.3. Vegetative compatibility grouping

Among the 60 strains, one was found to be self-incompatible. This strain, which did not form a heterokaryon with itself and with any other strain, could not be assigned to any VCG. Therefore, its status with respect to VCG could not be determined. The other 59 strains were assigned to 40 VCGs (Table 2). Most of them were single-isolate VCGs, 10 VCGs included two or more strains. Characterization of the 60 strains into VCGs was compared to the genotypic characterization. Grouping isolates on a vegetative compatibility basis appeared to be more discriminant than the PCR-RFLP method. Strains that grouped in the same VCG also grouped in the same IGS type.

Vegetative compatibility grouping of the strains was also compared to their trophic characterization. Analyses of variance were used to test for each growth parameter obtained on the four C sources whether means of the growth parameters were significantly different within VCGs and whether means of the growth parameters were significantly different between VCGs. The results of the ANOVAs indicate that there were significant differences ($P<0.05$) between growth parameters of isolates from different VCGs while there was no significant difference between growth parameters of isolates within VCGs. This means that isolates within a VCG are closer to each other than isolates from different VCGs regarding growth parameters. However, isolates from two or more VCGs may have similar growth parameters by chance. Therefore, multiple comparison of the means was performed with the Duncan test, which made it possible to distinguish the VCGs that were different from the others. Analogous results were found whatever the C source. However, for the sake of clarity only results obtained with glucose are presented (Table 3). On this substrate, VCG 27 grouped strains with the highest growth rate while strains having the lowest growth rate were found in VCGs 10, 19, 20 and 35, which were not distinguishable one from the other. Among r, P and K, the growth rate r was the most

discriminating parameter while P was the least discriminating.

4. Discussion

The results of this study indicate a substantial diversity among natural populations of *F. oxysporum* as reflected by both multiple growth characteristics and multiple VCGs. These results are consistent with the multiple IGS types found by Edel et al. [23]. Such a degree of intraspecific diversity within *F. oxysporum* populations has been suggested by several authors using genotypic features [18,23] but not on the basis of phenotypic characteristics. The absence of an efficient method to evaluate phenotypic traits was probably responsible for this failure. In this study, growth parameters of each isolate were estimated in synthetic liquid media containing a single C source. Glucose, maltose and sucrose are commonly used to grow fungi in culture media [34] and galactose was reported by Komada [24] to be a C source more specifically metabolized by *Fusarium* species than by other species of soil-borne fungi. Therefore, these C sources were expected to be used by all the isolates and to provide information on their specific growth parameters. Growth parameters of filamentous fungi are generally assessed by either dry weight measurements [35] or hyphal length measurements [36] but more rarely by optical density measurements [37]. Dry weight measurements are logistically difficult, time consuming and relatively expensive, and hyphal length measurements are tedious unless a video image analyzer can be used. Measuring OD changes in liquid medium using a microtiter plate reader is an accurate method to assess the growth kinetics of individual cell populations like bacterial populations [38] but can also be used to test fungal growth inhibitors [37]. Because (i) a positive relation was established between OD measurements and biomass content, (ii) no border effect was found, (iii) losses of water were very low and (iv) a good reproducibility was observed between replicates, OD measurement of *F. oxysporum* cultures with this method was considered a reliable method. The procedure allowed the collection of large sets of data and replicates for subsequent mathematical modelling.

Because of its simplicity, the logistic model has generally been accepted as the standard model for single-species population growth [39]. Fitting the logistic equation to each set of data allowed the characterization of the growth curves by three meaningful parameters: (i) r, the specific growth rate of the isolate on the given C source, (ii) K, the maximum population density of an isolate on a specified medium and (iii) P, the time at which the population density is equal to $K/2$. P is related to the length of the lag phase: the higher the P value, the longer the lag phase. Therefore, each set of three growth parameters was used to characterize a given strain and the PCA was then used to describe the relatedness between strains.

The dispersion of the strains along the two axes indicated a high level of diversity within a non-pathogenic population of *F. oxysporum* on the basis of growth parameter estimates on each of the four C sources. Some isolates exhibited very high growth characteristics while others had very low growth characteristics on each of the C sources tested. In all cases the growth rates measured in our study (from 0.058 h^{-1} to 0.353 h^{-1} on glucose) were similar to those already measured for other *Fusarium* species. Anderson and Solomons [34] reported that the maximum growth rate for *F. graminearum* strain IMI145425 was 0.28, 0.22 and 0.18 h^{-1} on glucose, maltose and maltotriose respectively as a C source, the r value decreasing with increasing length of the saccharide chain. Wiebe et al. [40] reported a specific growth rate of 0.26 h^{-1} for strain A3/5 of *F. graminearum* on glucose. Important changes in the specific growth rate values depending on the C source have also been reported for other fungi such as *Neurospora crassa* (0.2–0.58 h^{-1}) and *Gibberella fujikuroi* (0.13–0.21 h^{-1}) [41]. The growth parameters were estimated from growth kinetics in liquid medium containing a single C source. Therefore, they were higher than, and not directly comparable to the growth parameters in soil where C sources are multiple, rare, non-uniformly distributed and often non-soluble. Couteaudier and Steinberg [16] estimated the specific growth rate of *F. oxysporum* strains in disinfected soil to range between 0.008 and 0.016h^{-1}. Nevertheless, the estimated growth parameters are likely to reflect potential ecological traits of each strain or group of strains. Because similar results

were obtained with the four C sources, determination of growth characteristics using one C source (glucose for instance) would be enough to characterize new isolates.

Molecular tools have been widely used to characterize the diversity among pathogenic strains of *F. oxysporum* to determine genetic relatedness among *formae speciales* [21,42–44]. Less attention has been paid to the genotypic characterization of non-pathogenic strains [19]. More recently, Edel et al. [23] used a PCR-based procedure to characterize the 60 *F. oxysporum* strains used in this study. Eleven IGS types (IGS types 1–11) were found among the 60 strains. Therefore, to compare the phenotypic and the genotypic characterization, the IGS type number as defined by Edel et al. [23] was used as an external variable so that IGS types could be represented on the graphs of the PCAs. Apart from IGS type 4 and IGS type 9, grouping respectively isolates with low growth rates and K values and isolates with high growth rates and K values, the other IGS types grouped isolates whose characteristics were quite variable indicating that no direct relation could be drawn between phenotypic and genotypic characterization using these markers. One reason could be that the PCR-RFLP-based method was not discriminant enough compared to the trophic characterization.

Whether VCG could be related to the trophic or the genetic characterization was checked because vegetative compatibility has been frequently used to characterize populations or subpopulations of *F. oxysporum* [18–20,45]. Most of these studies revealed a limited number of distinct VCGs within a *forma specialis*. In contrast, attempts to group isolates of non-pathogenic *F. oxysporum* populations resulted in a large number of VCGs. Moreover, while a clear discrimination between groups is achieved when *formae speciales* are grouped on a vegetative compatibility basis, most VCGs among non-pathogenic populations of *F. oxysporum* are single-member VCGs, weak reactions linking VCGs can be observed, and intra-VCG incompatibility has been reported [18,46–50]. In this study 40 VCGs were found among 59 isolates. The percentage of strains that were single-member VCGs was 52% while Elias et al. [18] reported that 42% of the non-pathogenic strains of *F. oxysporum* isolated from symptomless tomato roots formed single-member VCGs. This proportion rose to 88% in the case of non-pathogenic *F. oxysporum* strains isolated from the rhizosphere of cotton plants [48]. For one of the strains, all pairings never gave rise to any, even weak, complementation. This isolate had to be considered a self-incompatible strain. The occurrence of self-incompatible strains at a very low frequency (4%) in *F. oxysporum* populations has already been reported [50].

To evaluate whether VCGs were related to trophic characteristics, only the growth parameters of the strains belonging to the 10 non-single-member VCGs were compared. Strains within VCGs had similar growth parameters while growth parameters may differ between strains from different VCGs. This indicates that each vegetative compatibility group has its own growth characteristics. In contrast to the PCR-RFLP method, which distinguished groups of strains among populations, the two other characterization methods used discriminated *F. oxysporum* at the strain level. Because isolates grouped in a given VCG had similar growth characteristics, a possible clonal origin for isolates within a VCG can be suggested. Vegetative compatibility assessment and genetic characterization gave correlated groupings although VCG appeared more discriminant than grouping obtained by PCR-RFLP analysis of the IGS region. The same VCG never occurred in two different IGS types. Altogether, the results reported here suggest that the non-pathogenic populations of *F. oxysporum* are composed of numerous genetically distinct isolated subpopulations. These subpopulations correspond to the vegetative compatibility groups which in turn could be considered the population units when looking at the ecology of *F. oxysporum*.

Comparing genotypic and phenotypic characterization as a whole, it is clear that both kinds of characterization are of importance. Genotypic characterization could indicate changes in the population structure due to any external factors such as agricultural practices or the cultivation of various plant species [51]. In contrast, phenotypic characterization could provide hypotheses to explain the population structure observed under a set of given conditions. Moreover, it could be used to screen for *F. oxysporum* isolates having specific growth characteristics

since these characteristics are related to biocontrol efficiency [17].

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