Sexual compatibility and oospore production of *Phytophthora infestans* on leaves and culture medium

G.R. TSOPMBENG NOUMBO[1], D.A. FONTEM[2] and C. ILOBA[3]
[1]Plant Biology Department, University of Dschang, Box. 67 Dschang, Cameroon.
[2]Plant Protection Department University of Dschang, Box. 208 Dschang, Cameroon.
[3]Crop Science Department, University of Nigeria, Nsukka, Nigeria.

ABSTRACT

Phytophthora infestans(Mont) de Bary, a destructive pathogen of potato and other solanaceous crops, is a heterothallic fungus that reproduces sexually in the presence of two compatible mating types. However, research on its sexual compatibility has not been investigated in Cameroon. To initiate this, isolates of the main hosts (huckleberry, potato and tomato) were analyzed for sexual compatibility on culture medium and oospore production on detached leaves. In the absence of reference (A1 and A2) isolates, compatibility tests were run using random crossing of the isolates. Of the 83 crosses made in culture medium, 28% were compatible and 72% were incompatible, while on potato and tomato detached leaves, 51% of the crosses produced oospores and 45% did not. These results suggest that the two mating types may be present in Cameroon. Oospore sizes were smaller than those reported. However, it is not known whether the oospores produced were as a result of mating of compatible thalli or selfing of compatible thalli and whether they are viable or not. Further research with mating type testers will improve the understanding of the sexual structure of *Phytophthora infestans* populations in Cameroon.

Key words: late blight, mating type, oospore, *Phytophthora infestans*, sexual compatibility

RESUME

Phytophthora infestans(Mont) de Bary, un pathogène destructeur de la pomme de terre et d'autres Solanacées, est un champignon hétérothallique qui se reproduit sexuellement en présence de deux types d'accouplement compatibles. Cependant, les recherches sur les types de comptabilité sexuelle de ce parasite n'ont pas encore été faites au Cameroun. C'est pour initier cela que des isolats provenant des principales plantes hôtes du mildiou (morelle noire, pomme de terre et tomate) ont été analysés pour leur compatibilité sexuelle en milieu de culture et la production des oospores sur des feuilles détachées. En l'absence des isolats de références(A1 et A2), les isolats utilisés ont été croisés au hasard. Des 83 croisements effectués en milieu de culture, 28% ont eu des réactions compatibles et 72% étaient incompatibles, alors que sur les feuilles, 51% des croisements effectués ont produit des oospores et 45% ne l'ont pas. Ces résultats suggèrent que les deux types compatibles de ce champignon peuvent être présents au Cameroun. Les dimensions des oospores produites étaient plus petites que celles reportées dans la littérature. Toutefois, il est difficile de savoir si les oospores observeés ont été formées à l'issue des croisements entre deux types de thalles compatibles ou un seul type compatible, et s'ils sont viables ou non. Des études antérieures avec les testeurs pourront nous permettre de mieux connaître la structure sexuelle de la population de *Phytophthora infestans* au Cameroun.

Mots clés: compatibilité sexuelle, mildiou, milieu de culture, *Phytophthora infestans*, type d'accouplement

INTRODUCTION

Phytophthora infestans, causal agent of late blight of potato and tomato worldwide is heterothallic and can reproduce sexually in the presence of the two mating types designated A1 and A2 (Gallindo. and Gallegly, 1960). Heterothallic fungi require interaction of two different thalli for sexual reproduction (Hawsesworth *et al.*, 1995). However, some strains of *P. infestans* are bisexual and are capable of self-mating (Mukalazi *et al.*, 2001; Cooke *et al.*, 2003). Either of the mating types is self-fertile when grown in isolation, but bisexual when it can form both antheridia and oogonia in the presence of a strain of the opposite compatibility type (Ko, 1978). Prior to the 1980s, the A2 was known to be restricted to Mexico, and only the A1 was spread worldwide and the fungus was thought to reproduce only asexually in other countries (Tooley *et al.*, 1986; Goodwin *et al*, 1995; Zhang *et al* 2001). However, after the 1980s, the A2 compatibility type was found in many countries of the world, including Europe, North Africa (Egypt), America and Asia (Shaw *et al.*, 1985, Drenth *et al.* 1993; Zhang *et al.* 2001).

Recent studies on sexual structure of the fungus conducted in Sub-Sahara African regions showed that Ugandan and Kenyan isolates of *P. infestans* produced oospores either through mating or selfing (Hohl, 1998; Mukalazi *et al.*, 2001). In South Africa, no evidence of the other mating type A2 was found on more than 600 isolates analyzed for sexual structure (Denner *et al.*, 2001). The mating type composition within population of Cameroonian isolates of *P. infestans* has not been investigated. This paper reports preliminary results on sexual compatibility and oospore production of the fungus.

EXPERIMENTS

Fungal inoculum

P. infestans inocula were harvested from 14 -21 days' pure cultures grown on V8 juice agar. Some were collected from freshly produced sporangia from the foliage of blighted garden huckleberry, potato and tomato. Sporangial suspensions (2 x 10^4sporangia/ml) were mixed in a 1:1 ratio and used for inoculation. The V-8 agar medium was prepared as described by Domsch *et al.*, (1990). Two hundred millilitres of V-8 juice was measured out. Then after filtering through a double-layer cheese cloth, 20 g of agar was added to the filtrate and 3 g of $CaCo_3$ to raise the pH. The volume was made up to 1,000 ml with distilled water before sterilizing at 0.5 atmosphere and 121°C in an autoclave.

Compatibility tests and oospores production on culture medium.

Compatibility tests were determined by random crossings of pure isolates of the fungus from garden huckleberry, potato and tomato. Two mycelial plugs of 4-mm-diameter each taken from two actively growing isolates of the fungus were placed 1 cm apart in the centre of Petri dishes containing V8 medium to which 150 ppm ampicillin and 100 ppm rifamycin had been added. These were incubated at 18 °C in the dark for 7 days. Crosses in which there was complete merging of mycelia were rated compatible, while those that had a clear line of demarcation were rated non-compatible. After 21 days, slides were prepared from the overlapped area of compatible isolates and examined for the presence of oospores, using a bright field microscope at x 400.

Oospore production on leaves

Healthy detached leaflets of potato (cv. Hydra) and tomato (cv. Roma) grown in pots in the screen house were thoroughly washed with water, blotted dry and placed abaxial side up on moist filter paper in 9-cm Petri dishes. Each leaflet was inoculated with 50 µl droplet of sporangial mixture. Dishes were incubated at 15°C for 18 hours in the dark. When a dark-brown lesion bordered by a light green to yellow halo had occurred on the leaflets, water was added to the plate in excess so that all leaflets were uniformly floating in the water (Cohen *et al.*, 1997). Two leaflets were used per treatment and the experiment was repeated once.

Microscopic observation

After 10 days, the surfaces were first washed with excess water to remove sporangia from the leaflets and then clarified in boiling ethanol for 5 minutes. After the ethanol treatment, they were again floated on water, blotted dry and a slide was used to lift the tissue and mount it on the glass surface on a drop of 50% glycerin before covering with a glass cover slip. The tissue was examined with a bright field microscope at x100 and x400 for oospore production.

RESULTS

Sexual compatibility and oospore production in culture

There was a significantly low frequency (28%) of compatible reactions and a high proportion (72%) of incompatible reactions in the 83 random crossings carried out in culture medium (Table 1). Most of the compatible reactions were obtained among isolates from

Table 1. Compatibility of *Phytophthora infestans* isolates in culture medium

Crossings	Compatible reactions	incompatible reactions	Total crossings
Huckleberry x huckleberry	1(11)	8(89)	9
Huckleberry x potato	5(5)	22(81)	27
Huckleberry x tomato	6(40)	9(9)	15
Potato x tomato	6(43)	8(57)	14
Potato x potato	2(17)	10(83)	12
Tomato x tomato	3(75)	1(72)	4
Total	23(28)	58(72)	81

* Figures in parenthesis are frequencies over the total number of crossings.

χ^2 = 126.29 df = 5 P< 0;001

Table 2. Frequency of oospores on potato and tomato detached leaflets

Pairings	Potato	Tomato	Potato and Tomato	Total.
Huckleberry x huckleberry	0(0)	1(50)	0(0)	2
Huckleberry x potato	2(25)	3(37.5)	2(25)	8
Huckleberry x. tomato	1(14)	1(14)	1(14)	7
Potato x tomato	6(33)	10(10)	4(22)	18
Potato x potato	6(29)	8(50)	5(31)	16
Total	15(29)	23(45)	12(23.5)	51

* Figures in parenthesis are percent over the total number of pairing

= 60.73 df = 8 P< 0;001

different hosts. A high frequency of compatible reactions was obtained whenever a tomato isolate was used in the crossing. Oospores produced as a result of compatible reactions were 12.5 - 17.5 µm (mean = 15.0 µm) in diameter (Fig. 1A).

Oospore production on detached potato and tomato leaflets

The results showed that 49% of the crossings did not produce oospores while 51 % produced oospores on potato or tomato leaves. The frequency of oospore production varied among both host plants used (X^2= 60.7, p< 0.001). A total of 45% of the crossings produced oospores on tomato leaves, 29% on potato leaves and 23.5 % on both hosts (Table.2). The oospores measured 15- 25 µm (mean = 20.0 µ) on tomato leaflets and 10 - 17.5 µm (mean= 12.5 µm) in diameter on potato leaflets (Figs 1B and C).

DISCUSSION

Results from all the pairings indicate that while sexual compatibility is rare among isolates of *P. infestans*, it is significant. Sexual compatibility ensures out-crossings of the isolates. Crosses between compatible strains are believed to yield oospores. According to Miller (2001), when compatible isolates come into contact, antheridia and oogonia of the two isolates of compatibility types can mate and sexual spores are formed. This sexual activity is stimulated by hormones released by a pair of compatible thalli (Judelson, 1997). The high proportion of incompatible reactions observed in culture media may preclude sexual reproduction in nature. Sexual incompatibility in *P. infestans* strains has been reported to lead to abortion and non-viable oospores (Erwin and Ribeiro, 1996).

Oospores produced on leaflets inoculated with a mixture of sporangial suspension were small in size compared to those found by Mukalazi *et al.* (2001) in Uganda. Both potato and tomato leaflets serve as good hosts for the formation of oospores though they were mostly found on tomato leaflets. This is in agreement with Cohen *et al.* (1997) who found that tomato generally supports more oospores than potato. Oospore formation indicates the potential for sexual reproduction of *P. infestans* and the probable existence of the

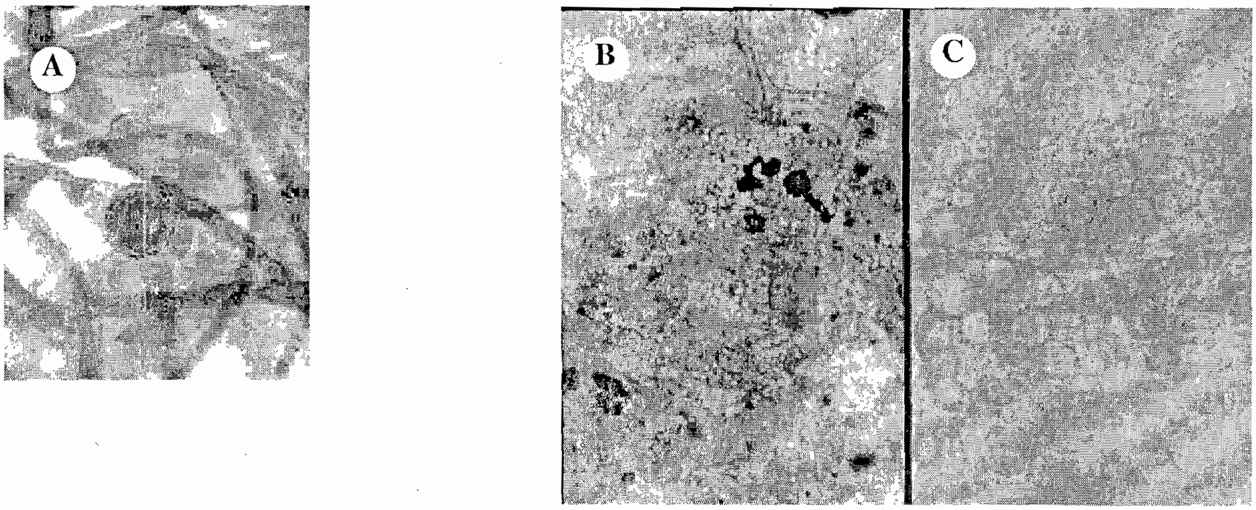

Fig.1. A-C. Oospores of *Phytophthora infestans*: **A,** oospore in culture medium at x 400 after 21 days at 18°C in the dark. **B** and **C,** oospores x 100 and x 400, respectively in a tomato cv (Roma) leaf coinoculated with (huckleberry x tomato) isolates after 10 days.

two mating types in Cameroon. Sexual reproduction in *P. infestans* leads to oospore production wherever the two mating types A1 and A2 are present in a population and come into contact inside a plant tissue (Zhang *et al.*, 2001). The presence of both mating types within the same field and consequently the occurrence of new genotypes has exacerbated disease problems, since some genotypes are resistant to phenylamide fungicide and at the same time more aggressive to their hosts (Chycoski and Punja, 1996; Fry and Goodwin, 1997; Ristaino, 2002). Metalaxyl-resistant isolates have been reported in populations of *P. infestans* in Cameroon (Fontem *et al*, 2001). Also, oospore progeny resulting from sexual cycle of the fungus can be more aggressive in consecutive crops in the field (Andersson *et al.*1998, Stromberg *et al.*, 1999).

Oospore production in plant material could have epidemiological implications. For instance, evidence of oospores infecting leaves that come into contact with the soil has been reported (Drenth *et al.*, 1995;Turkensen *et al.*, 2000). Although, the relative importance of oospores as an alternative means of survival in population of *P. infestans* is still not well established (Andrivon, 1995), their formation on leaves may have a strong impact on population structure of the fungus, when such leaves are not destroyed after harvest. Oospore occurrence on leaves suggests that tomato and potato leaves could serve as a major means of dissemination of these sexual spores from one season to the next. However, the ability of these oospores to survive in nature is uncertain, and most importantly, whether they are viable and can germinate and infect the hosts under field conditions is yet to be shown. As suggested by Cohen *et al* (1997), it is difficult to verify that oospores in infected leaves are responsible for resultant infection, because hyphae of both mating types will still be present and may also cause infection.

The absence of oospores in some of the crossings could be as a consequence of the presence of only one mating type of the isolates used, although oospore formation might be induced by interspecific pairings of the two mating strains (Boccas, 1981; Skidmore *et al.*, 1984).

Chi-square tests indicated that there was an uneven distribution of oospores in both hosts. It was not known whether the oospores observed were as a result of mating or selfing of similar mating types, and whether they are viable or not. However, sexual crosses within a single strain (homothallism) and between different strains (heterothallism) have been reported to result in the production of oospores (Cooke, 1994). The fact that mating-types isolates of *P. infestans* can be changed to the opposite type and that the self-fertile type can generate both mating types (Ko, 1994), suggests that these oospores may have originated from the opposite type or self-fertile type of the fungus. Further studies with mating-type testers (A1 and A2) will help us to understand the composition of *P. infestans* populations in Cameroon.

These results suggest that sexual compatibility in *P. infestans* exists in Cameroon and random crossings of isolates result in the production of small oospores on leaves. Whether these oospores are produced by mating or by selfing or whether they are viable or not is still under investigation.

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