European Journal of Plant Pathology **107:** 553–556, 2001.

Short communication

Production and germination of oospores of *Phytophthora infestans* (Mont.) de Bary in Morocco

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Accepted 22 February 2001

Key words: late blight, mating type, potato, tomato

Abstract

One hundred and eight isolates of *Phytophthora infestans* were collected from infected potato and tomato crops in the middle-north of Morocco during 1997–2000. Pairings of these isolates with tester isolates of mating type A1 and A2 revealed that 60% of the isolates were mating type A2 (65/108) and 40% were mating type A1. After 10 days incubation at 20 °C and a 16-h photoperiod, approximately 25% and 18% of the oospores produced *in-vitro* germinated in potato soil extract and potato root extract, respectively. Oospores were observed in potato leaf tissues in pairings that were fertile *in-vitro*. Maximum production of oospores was obtained in potato leaves of cultivars that were moderately susceptible (Desirée, Nicola) after 10 days of incubation at 15 °C and a 16-h photoperiod. These results confirm the presence of *P. infestans* strains that are sexually compatible under Moroccan climatic conditions. Production of oospores constitutes a threat for these crops because of the occurrence of recombinants with new virulences which may be difficult to control and as a consequence survival of oospores in absence of the host plant in the soil.

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most destructive diseases of cultivated potato and tomato. The population structure of this fungus is reported to have changed since the 1980s especially through the occurrence of the new strains of A1 and A2 (or both) mating types outside Mexico (Fry et al., 1992). In Morocco, Sedigui et al. (1997) found that isolates of *P. infestans* belonged to mating type A1. In this study, 108 isolates of *P. infestans* were collected from 19 fields of potato (101 isolates) and tomato (7 isolates) in the middle-north region of Morocco (Fés-Saïss) during the 1997–2000 cropping seasons. The pathogenicity of the isolates was tested by inoculating detached leaflets and tubers of Desirée potato cultivar and leaves of Daniela tomato cultivar with a suspension of 10-day-old sporangia. Mating types of isolates were determinated by pairing Moroccan isolates with tester isolates of mating type A1 and A2. The germination capacity of *in-vitro* produced oospores was determined. Suspension of oospores formed from different crosses were prepared according to modified procedure of Pittis and Shattock (1994) (without using the enzyme Novozym 234). Oospores were incubated on sterile plates containing different liquid media: sterile distilled water, horse and sheep dung infusion, potato soil extract, and potato root extract. The germination tests were conducted at 20 °C and 16-h photoperiod. The rates of germination of oospores were evaluated after 2, 3, 5 and 10 days intervals on different media. *In-vivo* sexual reproduction was conducted on leaf disks of four potato cultivars (Spunta, Desirée, Nicola and Kondor) according to Drenth et al. (1995).

Pathogenicity of the isolates

All the isolates were pathogenic. Inoculated leaflets developed typical late blight lesions after 2–5 days

incubation at 20 °C and 16-h photoperiod. In contrast, the reaction of the tubers incubated in dark at 18 °C was more variable. Some tubers did not exhibit any sign of infection while others developed varying degrees of rot. Isolates collected from tomato were less aggressive (smaller lesions, lower sporulation) than isolates from potato. However, most isolates from potato were very aggressive when tested on detached leaves of tomato cultivar Daniela.

Mating type of isolates

Pairings of Moroccan isolates with tester isolates of mating type A1 and A2 revealed that 60% of the isolates were mating type A2 (65/108) and 40% were mating type A1. All tomato isolates were A2. These pairing tests demonstrate a wide distribution of isolates of mating type A2. Strains of both mating type A1 and A2 were detected in the same field in a11 sampled areas (Table 1).

Rate of germination of oospores produced *in-vitro*

Oospores germinated after 2–3 days of exposure to light. Germination rates increased with the duration of exposure to light. The statistical analysis (Statistix Version 4.0) showed a significant difference ($P < 0.05$) between the media on which they were incubated. Rates of germination were 25%, 18%, 6%, 5%, and 4% after 10 days of incubation in potato soil extract, potato root extract, horse fresh dung infusion, sheep fresh dung infusion, and sterile distilled water, respectively.

In-vivo induction of sexual reproduction

Microscopic observation of lesions developed on leaf discs, inoculated with sexually compatible isolates, 7 days after incubation (20 °C and 16-h photoperiod), revealed abundant production of oogonia and antheridia in all crosses. Oospores were detected 10 days after incubation. Their number varied with the pairing and potato cultivar (Table 2). Statistical analysis (Statitcf Version 4.0) showed a highly significant effect ($P < 0.05$) of the pairing and the interaction of pairing × cultivar. Abundant production of oospores was obtained with moderately susceptible potato cultivars.

In the Saiss region of Morocco, *P. infestans* populations consisted of genotypes A1 and A2 with high frequency of A2 (65/108). This distribution is similar to that reported in Japan and Korea (Young et al., 1994), in Canada (Peters et al., 1998) and in the USA

Table 1. Origins and mating types of isolates

Areas	Number of isolates[a]	Dates of samples	Number of fields sampled[c]	Crops and varieties[e]	Infected organs analyzed	Mating type	
						A1	A2
Fés	25	12/01/1997 20/01/1997 14/01/1998 19/01/1998	4[d]	Tomato (Daniela) and Potato (Desirée)	Potato (leaflets and stems) Tomato (leaves and fruits)	3	22
Ain-Cheggag	23[b]	22/05/1997 24/05/1997 26/05/1997	5	Potato (Desirée; Nicola)	Leaflets and stems	2	21
Sefrou	4	11/01/1998 17/01/1998	1	Potato (Desirée)	Leaflets	2	2
Méknes	56	08/01/2000 11/01/2000 21/01/2000 28/01/2000	9	Potato (Desirée)	Leaflets	36	20

[a] Within each field, each isolate was collected from a ramdomly sampled plant from a particular tissues.
[b] Two isolates were self-fertiles but this self-fertility disappeared after two or three successive sub-cultures.
[c] Number of isolates ranged between 2 and 14 by field.
[d] Two potato fields and two greenhouses of tomato.
[e] Seeds imported from Europe.

Table 2. Evaluation of *in-vivo* fertility in three crosses on four potato cultivars

Crosses	Number of oospores formed/1 cm^2 of leaf tissue*			
	Desirée	Nicola	Kondor	Spunta
P6F17(A1) × S2F6(A2)	714 ± 217 a	417 ± 135 a	231 ± 137 a	92 ± 71 a
P6F16(A1) × S3F25(A2)	396 ± 152 b	347 ± 171 ab	321 ± 34 b	74 ± 31 b
S2F19(A1) × S2F1(A2)	202 ± 82 c	336 ± 125 ab	195 ± 75 c	0 c

Values followed by the same letters within each data column are not significantly different at the 5% probability according to the LSD test.
*Values are the means of 20 replicates.

(Fraiser et al., 1999). In Africa, the A2 mating type was detected only in Egypt (Shaw et al., 1985) and in South Africa (Andrivon, personal communication). All the tomato isolates, were A2. This suggests that exchanges between pathogen populations of potato and those of tomato are limited in this area as it has been proved for the relative pathogenic specificity showen in the test of pathogenicity. Likewise in France, Lebreton and Andrivon (1998) found strains of A2 mating type collected essencially on tomato. In Morocco, each year almost 36% of the potato seed tubers are imported from Europe (Anonymous, 1998; 1999). Since mycelium hibernating in the seed tubers constitutes one of the most important sources of primary inoculum of potato late blight, these may bring with them the two mating types which co-exist in many European countries (Drenth et al., 1993). The co-existence of these two forms in the same field could result in sexual recombination in Morocco. However, the microscopic observations of naturally infected host tissues did not reveal any presence of sexual organs. The date presented here agree with the observations of Drenth et al. (1995) and Hanson and Shattock (1998) that maximum production of oospores occurred in the foliage of the moderately susceptible potato cultivars. However, Cohen et al. (1996) found that host genotype had no significant effect on oospore formation. In the present study, potato soil extract is the appropriate stimulus for germination of oospores as pointed out by Medina and Platt (1999). The rates of germination obtained in different media are relatively higher than those of Pittis and Shattock (1994) who found germination levels ranging from 0 to 15%, but they are similar to those observed by Medina and Platt (1999) except for potato root extract. Nevertheless, oospore germination levels obtained here may be sufficient to induce infection. Further studies are needed to verify whether sexual reproduction occurs in Morocco and to examine the contribution of oospores to late blight epidemics.

Acknowledgements

We thank Professor M. Boulif, of the National School of Agriculture, Meknes, and Professor N. Amakhmakh of Al-Akhawayn University, Ifrane, for critically reading and correcting the manuscript and Dr D. Shaw of School of Biological Sciences in Gwynedd, U.K. for suppling the tester isolates of mating types A1 and A2.

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