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Phytophthora mirabilis, a new species of *Phytophthora*

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Abstract. – Based on host range, and on nutritional, biochemical and morphological criteria a new species of *Phytophthora, P. mirabilis,* is proposed. *Mirabilis jalapa* L. is the only natural host of this new species known so far.

Introduction

During the summer of 1954 SERVIN (10) found a *Phytophthora* pathogenic on wild *Mirabilis jalapa* L. (Nyctaginaceae) in the area of Chapingo, Mexico. This pathogen had larger sporangia and was more susceptible to malachite green (10) than *P. infestans.* In addition, it was not able to infect potatoes. At that time the differences were not considered important, particularly since the *Mirabilis* isolates formed oospores when paired with *P. infestans.* Later, FOURTON (3) using 24 isolates from *M. jalapa* found two mating types present and confirmed the inability to infect potato (*Solanum tuberosum* L.) and also the inability of *P. infestans* to infect *M. jalapa.* Mostly because of the ability of these strains to form oospores with *P. infestans* in paired cultures they were considered in both studies a variety of *P. infestans,* and later cultures from *Mirabilis* were listed as *P. infestans* in the collection of the Commonwealth Mycological Institute, Kew, England. In nutritional studies HOHL (5, 6) observed that a strain from *M. jalapa* grew substantially on a medium containing nitrate as the only nitrogen source, a behaviour at variance with other isolates of *P. infestans* tested. These results strengthened the idea that this pathogen of *M. jalapa* was distinctly different from *P. infestans,* particularly in view of their different host range. In this paper we present evidence which we believe necessitates the erection of a new species of *Phytophthora.*

Materials and methods

Isolations. – Twelve isolates were obtained from *Mirabilis jalapa* growing within an area of about 5 km^2 around Texcoco in the Central Plateau of Mexico, appr. 2220 m above sea level.

Colony growth and morphology. – The twelve isolates were characterized by determining their colony type on the two following natural media: (1) Potato dextrose agar (PDA), and (2) a combination of oat meal and V-8 medium (O-V8). For the latter 30 g of oat flakes were boiled in 400 ml of water. After filtration and centrifugation 100 ml of a clear supernatant was obtained. Separately, 150 ml of V-8 juice (Campbell Soup Corp.) were mixed with 2 g of $CaCO_3$, centrifuged and 100 ml of the supernatant combined with the 100 ml of cleared oat extract. The mixture was diluted to one liter with distilled water. 15 g of agar (Difco) were added before autoclaving.

Mating type. – The mating type was determined by pairing each isolate with an A1 mating type of *P. infestans*. Later the determinations were confirmed by pairing each isolate with one of the isolates from *M. jalapa* behaving as A2 type.

Pathogenicity. – Ten isolates from *M. jalapa* and 7 isolates of *P. infestans* from potato were inoculated onto detached leaves of potato cv. Patrones, tomato cv. Ace, and *M. jalapa*. The isolates from *M. jalapa* were also inoculated on detached leaves of potato cv. Atzimba, *Solanum ehrenbergii* RYDBERG, *S. stoloniferum* SCHLECHT. & BOUCHE, and *S. nigrum* L. Two drops of a sporangial suspension were applied to the anatomically lower side of each leaf and the leaves were placed in moist chambers and kept at about 20 C for 6 to 8 d before the reactions were scored.

Morphology of the isolates. – Dimensions of sporangia, oospores, oogonia, and antheridia were measured. The sporangia originated from infected leaf material. The sexual organs were taken from a mating of *Mirabilis* isolates M. 5 × M. 8 on O-V8.

Nutritional traits. – Nutritional characteristics of 6 isolates from *M. jalapa* were determined according to HOHL (6). Briefly, the isolates were grown on 5 media differing from each other mainly by their nitrogen sources. P-1L is the most complex medium containing vitamins, a purine base and trace elements in addition to a mixture of amino acids. Medium P-3 has asparagine, medium P-4 nitrate and medium P-5 ammonium ions as nitrogen source. The letter L (e. g. P-4L) indicates that the medium also contains 200 mg/L of lecithin known to improve growth of phytophthoras (5).

Enzyme production. – Isolates from *M. jalapa* and from potato were grown in the synthetic liquid medium P-1L (5) for appr. four weeks. The culture filtrates were then tested for the presence of two enzymes, β-glucosidase and esterase, using the chromogenic substrates p-nitrophenyl-β-glucoside and 4-nitrophenyl-acetate respectively.

Growth on selective media. – Strains from *M. jalapa* and potato were grown on two isolation media (SEL-1A and SEL-2A;

HOHL, unpublished) which are selective for *Phytophthora* species. Growth rates on the media were determined and compared with those obtained from the same medium lacking the selective compounds. The basal medium was prepared as follows: 200 g of rye was autoclaved in distilled water for 10 min and the suspension squeezed through a cheesecloth. The solution was diluted to one liter with distilled water and 5 g of glucose and 20 g of agar were added. Prior to autoclaving the following compounds in mg/L were added for SEL-1A and SEL-2A (values in parentheses) respectively: griseofulvin 20 (60) from SIGMA, nystatin 19 (65) from SIGMA, benlate 10 (30) with 50% active ingredient from MAAG, Dielsdorf, Switzerland, methoxypurine 5 (5) from SIGMA, hymexazol 0 (40) from SANKYO, Tokyo, Japan, rifamycin 30 (30) from CALBIOCHEM, nalidixic acid 5 (10) from SIGMA, 8-azaguanine 40 (80) and neomycin 30 (50), both from SIGMA.

Results

During field collection care was taken to avoid repeated isolation of the same strains. As may be seen from table 1 and figure 1 most isolates differ in at least one aspect from each other and thus are likely to represent different strains.

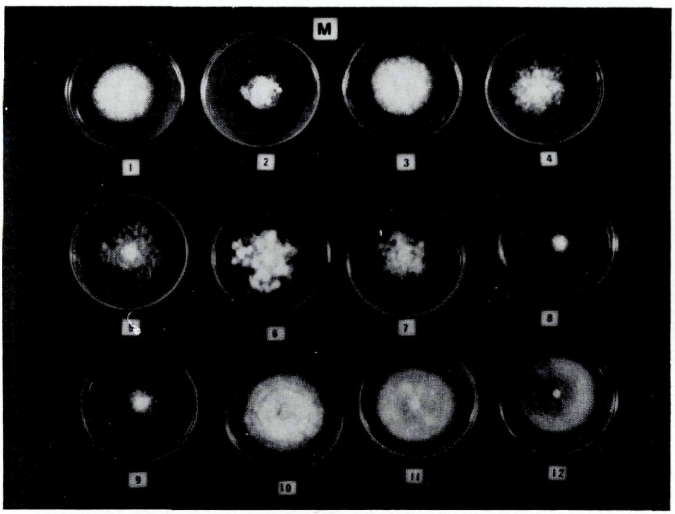

Fig. 1: Colony morphology (after 8 d on O-V8 agar) of the 12 isolates of *Phytophthora* from *Mirabilis jalapa* used in this study.

Table 1: Origin and properties of Mexican isolates of *Phytophthora* from *M. jalapa* and potato.

isolate/origin	growth PDA	(mm/d) O-V8	Colony type on O-V8 size (1)	shape (2)	mating type
from *Mirabilis:*					
M. 1 Horno-INIA	1.4	3.1	L	C	A1
M. 2 Horno-INIA	0.9	1.1	S	I	A1
M. 3 Horno-INIA	1.4	3.3	L	C	A2
M. 4 Cooperativo	1.2	2.0	M	I	A1
M. 5 Cooperativo	1.1	2.5	M	I	A1
M. 6 CIMMYT	1.4	2.5	M	I	A1
M. 7 CIMMYT	1.4	2.5	M	I	A1
M. 8 Rio Sn. Simon	1.0	1.0	S	C	A2
M. 9 Rio Sn. Simon	2.1	1.5	M	I	A1
M. 10 La Trinidad	2.0	4.3	L	C	A2
M. 11 Sn. Martin	1.3	3.8	L	C	A2
M. 12 Sn. Martin	1.6	4.0	L	C	A2
from *Solanum* (= *P. infestans*):					
P 1	1.6	4.9	L		
P 2	0.8	2.1	M		
P 3	2.2	4.7	L		
P 4	1.4	0	nd		
P 5	0.7	0.6	S		
P 6	0.4	0	nd		
P 7	0.4	0	nd		
P 8	0	0	nd		

nd = not determinable (no growth)
(1) S = up to 23 mm, M = 24 – 46 mm, L = 47 – 79 mm, after 8 d
(2) C = circular, I = irregular

Table 1 shows that growth on the O-V8 medium was generally better than on PDA for isolates from *Mirabilis* but that half the *P. infestans* strains from potato did not grow at all on O-V8. This indicates a difference in nutritional requirements of the two populations of isolates which will be further explored later. The highest growth rates observed were similar in both groups of isolates.

Of the 12 isolates from *M. jalapa* studied 7 were of mating type A1 while five were of type A2. Thus the ratio of A1 to A2 is 7 : 5, i. e. close to unity.

The pathogenicity tests revealed that the 10 isolates from *M. jalapa* included in the trials only infected their host of origin but not potato cv. Atzimba, tomato cv. Ace, *Solanum ehrenbergii, S. stoloniferum* or *S. nigrum*. The 7 potato isolates infected potato leaves and in some cases also tomato leaves but were unable to infect leaves of *M. jalapa*.

Table 2: Absolute and rel. growth rates (in % of P-1L) of *Phytophthora* strains from *M. jalapa* and of *P. infestans* strains from potato on 5 different media (see 6).

Strain	mm radial growth on P-1L	P-1L aa	P-3L asn	P-4 NO_3^-	P-4L NO_3^-	P-5L NH_4^+	Ratio P-4L/ P-5L
from *Mirabilis jalapa:*							
M. 4	0.9	100	199	142	136	84	1.6
M. 5	1.8	100	117	89	81	49	1.7
M. 6	2.3	100	94	22	38	38	1.0
M. 8	0.9	100	196	110	138	69	2.0
M. 9	1.2	100	155	36	47	65	0.7
M. 10	2.1	100	137	72	106	45	2.4
from *Solanum tuberosum:*							
of Central American origin							
Me 143	2.5	100	80	11	26	23	1.1
Me 144**	1.2	100	42	0*	0	0	nd
CR 133	1.0	100	0	0	0	14	nd
CR 134	1.8	100	0	0	0	0	nd
CR 135	1.5	100	0	0	0	0	nd
CR 136	1.5	100	0	0	0	0	nd
of European (USA) origin							
CG 96	1.5	100	157	36	36	100	0.4
223/33	4.1	100	32	0	0	13	nd
131.558 (USA)	2.2	100	46	11	16	21	0.8
B 25	2.9	100	0	0	0	0	nd
B 130	1.9	100	0	0	0	0	nd
78/1	2.2	100	100	20	25	65	0.4

nd not accurately determinable (growth too low)
* 0 means no growth or growth below 10%
** from tomato leaves
Me of Mexican origin
CR of Costa Rican origin

Table 2 shows that the nutritional patterns of all six isolates from *Mirabilis* were generally similar but differed from that typical for *P. infestans*. In particular the isolates from *Mirabilis* grew fair to well on nitrate nitrogen (media P-4 and P-4L) whereas isolates of *P. infestans* did not. Growth rates of the European isolates on P-1L and occasionally on other media were somewhat better compared to those from Central America. However, this difference is probably not an inherent difference but due to the long subcultivation on artificial media of the European but not of the Central American strains.

Table 3 records the enzyme activities determined for the two groups of isolates. With one exception only isolates from *Mirabilis* possessed esterase activity and their β-glucosidase activities clearly exceeded those of the potato isolates.

Table 3: Mycelial dry weight and extracellular enzyme production by *Phytophthora* isolates from *M. jalapa* and *P. infestans* isolates from potatoes.

Isolate	Origin	dry weight in mg*	β-Glucosidase** µg/min .ml cf	.mg my	Esterase*** µg/min .ml cf	.mg my
from *Mirabilis:*						
CMI 141.568	ME	17	27.0	7.8	4.8	1.4
M. 4	ME	81	3.5	2.2	1.1	0.6
M. 5	ME	71	2.6	1.9	1.0	0.7
M. 6	ME	118	2.7	1.2	0.5	0.2
M. 8	ME	128	4.0	1.6	0.9	0.4
M. 9	ME	130	5.0	1.9	1.6	0.6
M. 10	ME	92	2.0	1.1	1.3	0.8
from potato (tomato):						
S	CH	153	6.1	1.8	0	0
223/33	CH (?)	77	0.4	0.2	0	0
CMI 131.558	USA	53	0.4	0.4	0	0
B 25	UK	134	0.5	0.2	0	0
B 130	UK	168	2.1	0.6	0	0
36 (tomato)	I	165	0.8	0.2	0	0
78/1	UK	140	2.2	0.8	1.9	0.7
CG 96	?	166	1.0	0.3	0	0

Origin: ME Mexico, CH Switzerland, USA United States of America, I Italy, UK United Kingdom

* per 50 ml of culture filtrate, after appr. 4 weeks

, * enzyme activities of the culture filtrates in µg/min released dinitrophenol from the corresponding glucoside (**) and ester (***) respectively, expressed per ml culture filtrate (cf) and per mg of mycelial dry weight (my)

Table 4 summarizes the growth of the isolates from *Mirabilis* and from potato on two selective isolation media, SEL-1A and SEL-2A used for isolations of phytophthoras. SEL-2A which does not support substantial growth of typical *P. infestans* isolates did support fair to good growth of the isolates from *Mirabilis*. The apparent small differences in growth on SEL-2A between European and Central American isolates of *P. infestans* are probably due to the fact that the former had been cultured for years on artifical media while the latter were isolated within months before the tests were conducted. Since SEL-2A contains 40 mg/L of hymexazol which is toxic to typical *P. infestans* strains (HOHL, unpublished, 11) we presume that the differential action of SEL-2A is due to the presence of this compound which evidently does not stop the growth of the *Mirabilis* strains.

The 140 sporangia collected from leaves of *Mirabilis jalapa* had an average length of 33.7 µm (range 26.4 to 38.5 µm) while the 80 sporangia of *P. infestans* collected from *S. ehrenbergii,* potato cv.

Table 4: Relative growth rate in % of control of potato and *Mirabilis* isolates on two selective media, SEL-1A and SEL-2A.

isolates	origin	% growth on SEL-1A	% growth on SEL-2A
from *Mirabilis:*			
M. 4	ME	72	42
M. 5	ME	67	27
M. 6	ME	89	43
M. 8	ME	38	14
M. 9	ME	63	41
M. 10	ME	76	64
average		68	38
from potato (tomato):			
of Central American origin			
CR 133	CR, cv. Atzimba, leaf	8	0*
CR 134	CR, cv. Atzimba, leaf	35	0
CR 135	CR, cv. Atzimba, leaf	47	0
CR 136	CR, cv. Atzimba, leaf	23	0
ME 143	ME, potato, leaf	76	0
ME 144	ME, tomato, leaf	62	0
average		42	0
of European (USA) origin			
223/33		54	14
131.558	USA	42	12
B 25		56	24
B 130		42	0
average		48	12

* no growth or growth below 10% of controls
CR of Costa Rican
ME of Mexican origin

Atzimba and from the cultures on agar medium averaged 28.8 µm (range 22.0 to 39.6 µm). Isolates from *Mirabilis* were not only larger but also had a l/b ratio of 1.9 compared to 1.7 for those from potato. The 20 sex organs and oospores measured had an average size of 22 µm for the oospores, 26.4 µm for oogonia, and 13.8 × 11.8 µm (length × width) for antheridia.

Discussion

The apparent lack of an important or perhaps of any common host is evidence that isolates from *M. jalapa* must be considered different from the *P. infestans* isolates of potatoes even though the

two fungi are found in the same geographical area of Central Mexico. This host-related ecological isolation of the two populations exposes them to different evolutionary pressures as indicated by the morphological and physiological differences noted in the present and previous reports (3, 6, 10). The presence in this heterothallic species of the mating types in a ratio of approx. 1 : 1 provides optimal conditions for genetic recombination within the population, and the considerable differences found among the 12 isolates from *Mirabilis* coexisting in a relatively small area of 5 km^2 may be considered good evidence for the existence of such an efficient sexual process. The situation is quite different when one considers opposing mating types from *Mirabilis* and from potato. Here, the lack of a common host efficiently prevents breeding between the two populations.

An extensive nutritional evaluation of over 60 strains of *P. infestans* (6, table 2; HOHL, unpublished results) has shown that with rare exceptions members of this species are not capable of assimilating nitrate nitrogen to a substantial degree, i. e. do not grow adequately on the P-4L medium (6). Any deviation from this pattern must be considered important. Since all six isolates from *Mirabilis* tested showed a fair to good capacity to use nitrate as sole nitrogen source this nutritional property appears to represent an important and easily verifiable trait for distinguishing the two groups of isolates.

The differences with regard to extracellular enzyme production and growth on the selective medium SEL-2A may be useful additional criteria for distinguishing these two populations. This is noteworthy since more extensive studies on the general usefulness of extracellular enzyme production as a tool for species separation in *Phytophthora* have not been successful (7; HOHL unpublished results). As shown in this study and others (1, 2, 4) there are instances, however, where nonmorphological criteria provide useful taxonomic criteria for characterizing some species of *Phytophthora.*

The distinction of the two groups of isolates as discussed so far has been based on host range, nutritional and biochemical characters. It can be further substantiated using more traditional morphological parameters. The sporangia of the *Mirabilis* group are larger (34 versus 29 µm) and the oospores and oogonia are smaller (22 and 26 µm versus 30 and 38 µm respectively, see 9, 12) than those of *P. infestans,* In addition, the sporangia of the *Mirabilis* isolates are predominantly ellipsoid and have a higher l/b ratio compared to those of *P. infestans* (1.9 and 1.7, respectively).

The *Phytophthora* isolates from *Mirabilis* belong to the taxonomic group IV of WATERHOUSE (12), but differ from any of the species described in this group (8). They differ from *P. colocasiae,*

P. hibernalis, P. ilicis, and *P. melonis* by their compound sympodial sporangiophores with typical basal swellings, a trait they only share with *P. infestans* and *P. phaseoli.* From *P. phaseoli* they may be differentiated by their heterothallism while the differences to *P. infestans* have been enumerated and discussed above.

Based mainly on the complete disparity of host range and the nutritional, biochemical and morphological differences observed we propose the erection of the new species *Phytophthora mirabilis* for the isolates from *M. jalapa.* A description of the new species is given below.

Phytophthora mirabilis J. GALINDO & H. R. HOHL, spec. nov.

Coloniis mycelialibus in Secale vel Avena-V8 agaro bene crescentibus, nitrata assimilantibus. Cultura minima ad 7 C, optima ad 21 C et maxima ad 27 C. Hyphae eseptatae et copiose ramosae, 4.4–7.7 µm diam. (ad maximam partem 5.2 µm). Sporangiophori aerii in agaro ramis composito-sympodialibus et indeterminatis, cum tumoribus in loco sporangiis emergentes. Sporangia semipapillata, ellipsoidea, saepe basi attenuata, caduca cum pedicella brevi, valore medio 3.4 µm longa (variatione 2.6–3.9 µm), ratione longitudinis/latitudinis 1.9, germinantia directe tubo germinativo vel indirecte cum zoosporis 5–11. Antheridia amphigyna, valore medio 13.8 µm longa, ratione longitudinis/latitudinis 1.1. Oogonia laevitunicata, valore medio 26.4 µm diam., basi attenuata. Oosporae laevitunicatae, incoloratae, valore medio 21.3 µm. Segregatis heterothallicis, typis conjugationis A1 et A2 inventis circ. aequinumerosis. Hab. solum in *Mirabilis jalapa* nota. Mexico (typus, M. 10, CBS).

Mycelial colonies grow well on rye or oatmeal-V8 agar and are capable of using nitrate-N as sole nitrogen source. Minimum growth at about 7 C, optimum at 21 C and maximum at 27 C. – Hyphae nonseptate and freely branching, with hyphal diameters 4.4 to 7.7 µm , mostly 5.2 µm. – Sporangiophore aerial on agar with compound-sympodial and indeterminate branches, with swellings where sporangia emerge. – Sporangium semipapillate, ellipsoid, frequently with tapered base, caducous with short pedicel, on average 3.4 µm long (range 2.6 to 3.9 µm) with a length/width ratio of 1.9, germinating directly with germ tubes or indirectly with 5–11 zoospores. – Antheridia amphigynous, average length 13.8 µm, ratio of length/width 1.1. – Oogonia smooth-walled, average diameter 26.4 µm, with tapered base. – Oospores smooth-walled, colorless, average diameter 21.3 µm. – Isolates heterothallic with the A1 and A2 mating types found in a ratio of appr. 1 : 1.

Known host range restricted to *Mirabilis jalapa* L.

Type specimen isolate M. 10 has been deposited with CBS.

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