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Metalaxyl resistance, mating type and pathogenicity of *Phytophthora infestans* in Uganda

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Abstract

A total of 81 isolates of *Phytophthora infestans* (Mont.) de Bary were recovered from late blight infected samples collected from different areas of Uganda in 1998/1999. They were analyzed for their resistance to metalaxyl fungicide, mating types, and cross infection between potato and tomato hosts. Sensitivity to metalaxyl was determined by growing isolates on 10% V8 medium amended with 0, 5 and 100 μg/ml metalaxyl. Overall 44.4% of the isolates tested were resistant to metalaxyl, 23.5% were intermediate and 31.2% were sensitive. Mating type was determined on 80 isolates using an A1 (1724) isolate and by growing the same isolates in pure culture (selfing). Fifty percent of the isolates produced oospores by matings and selfings, 22.5% by mating only and 10% by selfing only; 18% did not produce oospores in the two tests. Twenty seven of the *P. infestans* isolates from potato-infected tomato. The majority of these isolates were highly resistant to metalaxyl and produced oospores by either mating or selfing or both.
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1. Introduction

Until recently, populations of the late blight fungus, *Phytophthora infestans* (Mont.) de Bary outside Mexico contained only the A1 mating type. This meant that except for within Mexico, considered the place of origin of this pathogen (Niederhauser, 1991), the fungus could not complete its sexual life cycle. Considerable evidence suggests that about 20 years ago new populations of the fungus containing both A1 and A2 mating types escaped Mexico and spread around the world (Fry et al., 1992). These isolates are rapidly replacing the original lineages in many regions (Fry et al., 1993). They carry the potential to complete their sexual life cycle, which includes formation of oospores that enable the fungus to survive for long, and appear to have higher levels of virulence and high frequencies of resistance against the widely used fungicide metalaxyl (Deahl et al., 1995; Goodwin et al., 1996).

Except for one report of the occurrence of the A2 mating type in an Egyptian population (Shaw et al., 1985), there are no definite indications of occurrences of new biotypes in Africa. However, the increased occurrence of devastating late blight epidemics, coupled with reduced fungicide sensitivity (Fry et al., 1993) suggest the possible existence of new *P. infestans* populations in the continent. Another evidence from studying the population structure of *P. infestans* in North America suggests that pathogenic aggressiveness on tomato occurs within the potato-aggressive populations, and the tomato-aggressive genotypes are aggressive on potato (Legard et al., 1995). It is not known in Uganda whether tomato and potato are attacked by the same *P. infestans* population. Strategies to reduce their potential destructive effects on potato production must be developed. To do this it would be helpful to acquire more knowledge on the population structure of the fungus in the region. This study was conducted to characterize *P. infestans* populations in Uganda with respect to the possible occurrence of the A2 mating type, pathogen resistance to metalaxyl, and pathogenicity of the fungus on potato and tomato hosts.

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2. Materials and methods

2.1. Sample collection and laboratory isolation of P. infestans

Samples of potato leaves infected with late blight were collected from 136 sites in four districts of Uganda, namely; Kisoro, Kabale, Mbarara and Mbale. This was done in May and November 1998, and May 1999. At all sites single samples were collected by arbitrarily picking infected leaves from each field. Sampling was done every 15–20 km of a road system across each district. Infected leaf tissue was placed under a thin, surface disinfected slice of potato tuber of variety Victoria (CIP381381.20) and incubated at 17–20°C for seven days to allow for mycelial growth through the potato slice.

Mycelia from the surface of the slice were picked and aseptically transferred to selective (SEL) V8 medium (Hohl, 1991) to avoid bacterial contamination. The plates were incubated at 17–20°C, and after seven days sporangia were transferred to 10% V8 medium for subsequent studies. Vegetable oil (1 ml/l) was added to the two media to promote sporulation of *P. infestans* (Klemmer and Lenney, 1965). Twenty five isolates (Kisoro, 3; Kabale, 3; Mbarara, 13; Mbale, 6) were recovered from the May 1998 collections, 26 (Kisoro, 3; Kabale, 15; Mbarara, 5; Mbale, 3) from November 1998 and 30 (Kisoro, 2; Kabale, 21; Mbarara, 2; Mbale, 5) from the May 1999 collections; a total of 81 isolates. Few numbers of *P. infestans* isolates were recovered from the samples, because in some cases the fungus did not grow through the potato slice as a result of bacteria contamination or death of spores in the samples from unexpected fungicide sprays on the crop prior to collection.

2.2. Metalaxyl resistance test

Sensitivity to metalaxyl was determined for the 81 isolates using 10% V8 medium supplemented with 90.6% technical grade metalaxyl (Ciba, Switzerland), at concentrations of 0, 5 and 100 μg/ml metalaxyl, replicated 4 times. Metalaxyl was prepared as a 100 mg/ml stock solution in pure dimethylsulfoxide (DMSO) and appropriate volumes were added to molten (40°C) agar after autoclaving to bring it to the desired concentrations. To avoid the effect of different DMSO concentrations on growth of *P. infestans*, appropriate amounts of DMSO were added to the control and the 5 μg/ml media so that each treatment had the same amount of DMSO. Agar plugs approximately 5 mm in diameter were cut from the edge of a 10–14 day old V8 colony and placed at the center of each metalaxyl amended plate. After incubation for 21 days at 17–20°C, diameters of each colony were measured. For each season collection, the procedure was repeated three times and data collected subjected to analysis of variance (ANOVA) using general linear model (GLM) procedure of the Statistical analysis software (SAS) package (SAS, 1987).

Although the ANOVA indicated significant difference ($P \leqslant 0.05$) among the repeat tests, the data were pooled for ease of data presentation, and the trends were generally consistent.

Estimates of the growth rate relative to the control (0 μg/ml metalaxyl) were also calculated. Subsequently, isolates were separated into groups depending on their sensitivity to metalaxyl following the procedures used at the International Potato Center (Forbes, 1997). Sensitive isolates were those with growth $< 40\%$ of the control at 5 and 100 μg/ml; intermediate isolates had a growth $\geqslant 40\%$ of the control at 5 μg/ml but less than 40% of the control at 100 μg/ml; resistant isolates had growth $\geqslant 40\%$ of the control at 100 μg/ml. The counts of *Phytophthora infestans* isolates in the metalaxyl reaction groups were then analyzed using the GLM procedure-three-way contingency table analysis where, season, districts and metalaxyl reaction groups were the factors.

2.3. Mating-type test

Mating type was determined on 80 of 81 isolates (1 isolate was lost due to contamination) by placing a plug of an unknown isolate on one side of a petri dish containing 10% clarified V8 medium with a plug of a known A1 isolate (1724). The A1 isolate originally from Kenya was obtained from Gregory Forbes of the International Potato Center (CIP), Ecuador. Each isolate was replicated in 4 plates. The same set of isolates were also grown in pure cultures (selfing). The plates were incubated at 17–20°C for 21 days after which formation of oospores were observed under a binocular microscope at low magnification (40×) which covers an area of about 35% of the plate. Four to six areas for each of the plates were examined in search of oospores. Counting of oospores was done by turning the plates upside down and focusing on the bottom of the plate. Since there were much variation in the oospore counts per plate, and a number of plates had zero oospore counts, data were subjected to square root transformation ($x + 0.5$) to normalize the variance. The transformed data were later subjected to Analysis of variance (ANOVA) using general linear model (GLM) procedure of the SAS statistical package (SAS, 1987). Each experiment repeat was analyzed separately. The isolate counts in the oospore production categories, i.e., non oospore producers, oospores producers by selfing only, oospore producers by mating only, and oospore producers by both mating and selfing were analyzed using GLM procedure-three-way contingency table analysis. Season, districts and oospore production categories were the factors.

2.4. Pathogenicity of P. infestans isolates

Pathogenicity of 61 isolates (20 isolates were lost due to contamination) from potato was tested on potato variety Victoria (CIP 381381.20) and tomato variety Maglobe, both of which have high level of susceptibility to late blight. Potato and tomato leaves were detached from 6–8 weeks old plants grown in greenhouse, and the younger, upper leaflets were used. Leaves were washed with sterile distilled water for about 20 min, and were later blotted slightly on paper towels to remove excess moisture. Bases of the leaflets were covered with pieces of moist cotton wool to reduce leaf desiccation. The leaves were then placed upside down (abaxial side up) into 9 cm plastic petri-dishes containing moistened filter paper. Two leaflets were placed in each petri-dish.

Inoculation of the leaflets was done following procedures described by Forbes (1997). Sporangial suspensions were produced from cultures by gently washing off the mycelium on the plate culture with sterilized distilled water. Sporangial concentrations were determined with a haemacytometer and adjusted to about 60,000 sporangia per milliliter. The sporangia were incubated at 4°C for 2 h to induce germination. Subsequently, three drops of 10 μl of sporangial suspension were applied to the mid-ribs of two leaflets. The inoculations were replicated in four plates, and incubated at 17–20°C for seven days. Subsequently, the leaflets were assessed for percentage leaf area blighted and lesion length. The experiment was repeated three times for the isolates tested.

The data on lesion length were transformed using square root transformation ($x + 0.5$) to normalize the variance. Square root transformations were used because some lesion length values were zero. Arcsine transformations were used on percent leaf area blighted. For each season, the experiment was repeated three times, and the data pooled for statistical analysis. The transformed data were subjected to analysis of variance (ANOVA) using general linear model (GLM) procedure of the SAS statistical package (SAS, 1987). Means of the transformed data were separated by the least squares. Since different isolates were studied each season, individual season's data are presented. An isolate was considered virulent on the host leaves when the mean sporulating lesions were $\geqslant$ 1 cm in length (Forbes et al., 1997).

3. Results

3.1. Metalaxyl resistance test

Differences in mean growth rates for the three metalaxyl concentrations were highly significant ($P = 0.001$). The addition of metalaxyl to culture medium significantly reduced growth rates in most of the isolates tested (Table 1). For the May 1998 collection, 11 *P. infestans* isolates were considered resistant to metalaxyl. The isolates had their mean percentage growth rates on media with 100 μg/ml of metalaxyl, relative to the control ranging from 32.4% for isolate 4002 to 76.6% for isolate 2002. From the November 1998 collection, eight *P. infestans* isolates were resistant to metalaxyl, of which isolates 3015 and 2022 had highest and lowest mean growth rates of 57.3 and 32.3%, respectively, on media with 100 μg/ml. In the May 1999 collection, mean growth rates for the 17 resistant *P. infestans* isolates ranged from 39.8% for isolate 2028 to 75.2% for isolate 2036 on media with 100 μg/ml metalaxyl relative to the control (Table 1). The growth rates of metalaxyl-resistant *P. infestans* isolates in this study, compare with the range of 52–83% growth rates of the metalaxyl insensitive *P. infestans* used in the study of 'multiple loci determining insensitivity to phenylamide fungicides in *P. infestans*' (Judelson and Roberts, 1999). Overall 44.4% (36 of 81) of the isolates tested were highly resistant to metalaxyl, 23.5% showed intermediate resistance and 31.2% sensitive (Table 2).

The number of *P. infestans* isolates that were highly resistant, intermediate and sensitive to metalaxyl did not vary with season, but varied with location from which they were collected (Table 2). Fifty percent (18 of 36) of highly resistant *P. infestans* isolates were from Kabale district with a mean of 6.0 resistant isolates per season, while Kisoro registered only 2.8%, with mean of 0.3. Mbarara and Mbale had about 22% of the highly resistant isolates, with means of 2.7 per season (Table 2). Although Kabale district had the most metalaxyl-resistant *P. infestans* isolates (18 of 39), Mbale district registered the highest percentage of 57.1% (8 of 14) from an individual district. Kisoro isolates comprised 15.8 % (3 of 19) of the intermediate isolates while Kabale and Mbale each had 26.3% and Mbarara 31.6 % (Table 2). Kabale district had the majority of the sensitive isolates (61.5%) while Mbale had the least 3.9%.

3.2. Mating-type test

With both selfing and crossing, the number of oospores produced varied significantly ($P < 0.05$) with the isolates tested. Among the isolates collected in May 1998 (Table 3), isolates 2002, 3008 and 4001 produced the highest number of oospores in matings, averaging 12.0, 26.0 and 17.5 oospores/plate, respectively. However, production of oospores was inconsistent (hence data for individual experiments are presented). For example, isolate 3008 produced 15 oospores in the first test, 12 in the second, but 51 in the third. In contrast, isolate 3012 produced 15 oospores in the first test, six in the second and 10 in the third. Isolate 3013 consistently produced the highest number of oospores in selfing and only few in matings. Isolate 1001, 3004, 3007 and 3011 did not produce oospores, whether by matings or selfing (Table 3).

Table 1
Mean[a] colony diameter (mm) after 21 days of incubation and percent (%) growth rates relative to control of *P. infestans* isolates at two metalaxyl concentrations[b,c]

May 1998				November 1998				May 1999			
Isolates	Control colony diameter in mm (0 μg/ml)	% growth rates (5 μg/ml)	% growth rates (100 μg/ml)	Isolates	Control colony diameter in mm (0 μg/ml)	% growth rates (5 μg/ml)	% growth rates (100 μg/ml)	Isolates	Control colony diameter in mm (0 μg/ml)	% growth rates (5 μg/ml)	% growth rates (100 μg/ml)
1002[d]	66.8 c	43.4 f	35.3 f	2009[d]	71.5 a	61.4 b	48.7 b	2026[d]	60.3 f	50.5 hij	49.3 fg
2001[d]	69.7 bc	64.4 b	71.0 b	2016[e]	69.9 abc	44.6 d	35.6 c	2028[d]	54.6 h	41.6 k	39.8 h
2002[d]	73.1 a	91.2 a	76.6 a	2017[e]	64.1 d	41.8 de	27.0 d	2031[d]	58.9 f	52.9 ghi	50.3 efg
3001[d]	68.8 bc	41.9 f	31.9 f	2021[d]	70.0 ab	55.4 c	48.6 b	2033[d]	66.6 bc	49.9 ij	55.7 def
3008[d]	68.5 bc	52.2 d	46.7 e	2022[d]	60.6 f	42.4 de	32.3 cd	2034[d]	66.0 bcde	58.4 efg	46.2 gh
3009[d]	71.3 ab	51.3 d	48.6 de	2023	66.9 cd	27.1 f	15.4 e	2035[d]	67.3 bc	76.5 ab	70.3 ab
3010[d]	55.1 e	50.9 d	57.9 c	3014	70.0 ab	19.4 g	15.6 e	2036[d]	67.0 bc	81.5 a	75.2 a
3012[d]	67.0 c	44.3 ef	35.1 f	3015[d]	67.5 bc	56.0 b	57.3 a	2037[d]	60.3 f	64.6 cde	56.5 de
4001[d]	70.0 bc	48.7 de	51.5 d	3016[e]	69.1 abc	38.7 e	17.6 e	2038[d]	66.3 bcd	75.1 ab	66.5 bc
4002[d]	67.0 c	43.9 f	32.4 f	3017[d]	69.5 abc	36.6 e	50.0 b	2039[d]	71.5 a	65.8 cd	44.6 gh
4006[d]	69.4 bc	58.6 c	57.0 c	3020[d]	70.2 ab	41.5 de	45.7 b	2040[d]	67.5 b	61.8 def	60.7 cd
1724[f]	59.8 d	15.5 g	11.7 g	4008[d]	70.2 ab	41.6 de	45.7 b	2042[d]	64.7 cde	49.2 ij	48.3 g
				4009[d]	68.4 bc	61.6 b	45.4 b	2046[d]	70.9 a	71.0 bc	71.3 ab
								3021[d]	63.7 de	57.1 fgh	57.2 de
								4010[d]	65.6 bcde	56.4 fghi	48.9 fg
								4011[d]	65.9 bcde	66.1 cd	62.2 cd
								4012[d]	57.0 gh	39.8 k	73.6 ab
								4013[e]	63.7 de	43.4 jk	28.0 i
								4014[e]	63.3 e	59.4 defg	28.9 i

[a]Colony diameter = measured diameter on media minus inoculating plug (5 mm).
[b]Isolate source: Kisoro = 1000 + , Kabale = 2000 + , Mbarara = 3000 + , Mbale = 4000 + .
[c]Within columns, means with the same letter(s) do not differ significantly at ($P \leqslant 0.05$) using LS means PDF.
[d]Highly resistant *P. infestans i*solates to metalaxyl, in at least one run of the experiment.
[e]Intermediary resistant *P. infestans i*solates to metalaxyl, in at least one run of the experiment.
[f]1724 is A1 isolate.

Of the November 1998 isolates, isolates 1007, 2017, 2018, 3016, 3017 and 3020 produced the largest number of oospores. Isolates 1007, 3017 and 3020 produced large number of oospores in both mating and selfing, but isolates 2006, 2008, 2013 and 2015 did not produce oospores. Among the isolates collected in May 1999, 3022 (data not presented) produced many oospores, but only with mating. Isolates 2030, 2036, 2046 and 3021 produced oospores in mating and selfing, but more oospores were produced with mating. For this season, isolates 2025, 2028, 2032 and 2033 did not produce oospores by mating or selfing. Overall, 82.5% of the isolates tested produced oospores in matings and selfings with more oospore production in mating (22.5%) than selfing (10%). For the 80 *P. infestans* tested for oospore production, 50% of the isolates produced oospores in both matings and selfings and 18% did not produce oospores in the two tests. Oospore production was not influenced by seasonal differences, but was influenced by the district of origin (Table 4). Although the greatest number of oospore producing isolates were produced by mating and selfing, Kabale district recorded the highest number of isolates in each of these categories. Interestingly, Kabale also had the largest number of isolates that did not produce oospores. All isolates from Mbale produced oospores.

3.3. Relationship between metalaxyl sensitivity and oospore production

The isolates used for metalaxyl tests were the same isolates used for oospore production. Ninety two percent (33 of 36) of the highly resistant *P. infestans* isolates produced oospores in cultures. On average the number of oospores produced by highly resistant isolates ranged from 1 to 50/plate. Of the 36 isolates, 11 (30.5% of metalaxyl resistant isolates) produced oospores by mating only and 20 (55.5%) produced oospores by both mating and selfing. Three metalaxyl-resistant isolates (8.3%) did not produce oospores in both tests (Table 5). Eighty three percent of the intermediary resistant isolates produced oospores, with 1–45 oospores/plate. Sixty seven percent produced oospores in both mating and selfing tests, 6% by selfing only, 11.1% by mating only

Table 2
Reaction of 80 *P. infestans* isolates from Uganda to metalaxyl collected during 1998–1999[a]

Months	District	Number of *P. infestans* isolates			
		Resistant	Intermediate	Sensitive	Total
May 1998	Kisoro	1	0	2	3
	Kabale	2	0	1	3
	Mbarara	5	5	3	13
	Mbale	3	2	1	6
	Total	11	7	7	25
November 1998	Kisoro	0	2	1	3
	Kabale	3	3	9	15
	Mbarara	3	1	1	5
	Mbale	2	1	0	3
	Total	8	7	11	26
May 1999	Kisoro	0	1	1	2
	Kabale	13	2	6	21
	Mbarara	1	0	1	2
	Mbale	3	2	0	5
	Total	17	5	8	30
	Total	36	19	26	81

[a]Resistant: growth on media with 5 and 100 μg/ml metalaxyl $\geqslant 40\%$ of 0 μg/ml; intermediates: growth on media with 5 μg/ml metalaxyl $\geqslant 40\%$ of 0 μg/ml; sensitive: growth on media with 5 and 100 μg/ml metalaxyl $\leqslant 40\%$ of 0 μg/ml.

and 16.7% did not produce oospores (Table 5). Among the metalaxyl sensitive *P. infestans* isolates, 69.2% produced oospores in cultures, with 1 to 20 oospores / plate. Five (19.2%) of the sensitive isolates produced oospores by mating only, 5 (19.2%) by selfing only, and 8 (30.7%) by both selfing and mating. Eight (30.7%) of the sensitive isolates did not produce oospores. Overall, irrespective of the metalaxyl sensitivity, most of the oospores were produced by the metalaxyl resistant isolates, followed by sensitive isolates; the least number of oospores produced was by intermediate category (Table 5).

3.4. Pathogenicity of *P. infestans* isolates on potato and tomato leaflets

The amount of leaf damage (lesion size and mean percentage leaf area blighted varied significantly ($P <$ 0.05) with the isolates, but results of the experimental repeats were consistent. Lesion length and percentage leaf area affected also varied significantly ($P \leqslant 0.05$) with the inoculated hosts (tomato or potato). All isolates tested caused disease on detached potato leaves.

For the May 1998 collection, the isolates that caused disease on tomato included, 2002, 2003, 3008, 4001,4001, 4002, 4003, 4004 and 4006. The lesion length ranged from 9.0 mm for isolate 3010 to 22.5 mm for isolate 4002, and the percentage area affected ranged from 7.8 to 32.5% for isolates 3010 and 4002, respectively. Isolates 4002 and 4004 caused significantly ($P \leqslant 0.05$) more disease on the detached tomato leaves than on potato (Table 6).

Of the 18 isolates tested for pathogenicity on potato and tomato from the November 1998 collection, 11 isolates caused disease on tomato leaflets. Isolates 2009, 2014, 2015, 2016, 2018, 2021 and 3020 caused lesion of more than 10 mm long, and were, therefore, considered to have infected tomato. Overall, however, lesion lengths and percentage infected area on tomato leaflets were lower than those on potato leaflets. In May 1999 collection, 12 isolates were pathogenic to tomato leaflets with isolate 2046 causing the highest lesion length of 23.8 mm and 2038 the least length of 12.5 mm.

Pathogenicity of the *P. infestans* isolates was also related to metalaxyl sensitivity, oospore production and origin of the isolate. Of the 61 *P. infestans* isolates used in the pathogenicity, 14 (22.9%) that infected detached tomato leaves were highly resistant to metalaxyl (Table 7), 5 (8.2%) were intermediates, and 8 (13.1%) were sensitive. All isolates caused disease on potato leaves but isolate 2009 caused a lesion size of less than 10 mm.

Eighteen (29.5%) of the isolates that infected tomato leaflets produced oospores by both mating and selfing, 2 (3.3%) in selfing only, 3 (4.9) by mating only, and 3 (4.9%) did not produce oospores (Table 7). Irrespective of oospore production, all isolates caused disease on detached potato leaflets. Considering location, 16 (26.2%) of the isolates that were pathogenic on tomato leaves originated from Kabale, and 7 (11.5%) and 3 (5%) were from Mbale and Mbarara, respectively. Only one isolate from Kisoro infected tomato leaves. Overall, the largest number of isolates that infected tomato leaves were from the highly resistant metalaxyl group of *P. infestans* isolates, and produced oospores by mating or selfing (Tables 7). Most of these isolates originated from Kabale (16 of 27) and Mbale (7 of 27) few were from Mbarara (3 of 27) and Kisoro (1 of 27).

4. Discussion

The most unexpected aspect of this study was the relatively high proportion of metalaxyl resistance among the *P. infestans* isolates collected in the major potato growing districts of Uganda. In this country there is only moderate Ridomil (56% Mancozeb and 7.5% metalaxyl) application to control late blight, largely due to economic restriction. In other parts of the world, resistance to metalaxyl and other related compounds has developed rapidly in populations of *P. infestans* after the use of metalaxyl (Davidse et al., 1983; Deahl et al., 1995). According to some authors, a greater frequency of resistance to metalaxyl occurs among the new biotypes having both A1 and A2 mating types (Fry et al., 1993; Erwin and

Table 3
Oospore production *P. infestans* isolates in selfing and mating[a,b]

Isolate	Mean number of oospores per plate							
	1st run		2nd run		3rd run		Mean	
	Mating	Selfing	Mating	Selfing	Mating	Selfing	Mating	Selfing
May 1998								
1001	0	0	0	0	0	0	0.0	0.0
2002	6 b	0	15 a	0	14 b	2	12.0 b	1.0
3004	0	0	0	0	0	0	0.0	0.0
3007	0	0	0	0	0	0	0.0	0.0
3008	15 a	3 b	12 ab	1	51a	2	26.0 a	2.1
3011	0	0	0	0	0	0	0.0	0.0
3012	15 a	0	6 c	0	10 b	1	10.3 b	0.3
3013	2	44 a	1	10 a	9 b	47 a	4.1	34.0 a
4001	7 b	4 b	8 bc	2	39 a	5	17.5 a	3.3
1724[b]	0	0	0	0	0 d	0	0.0	0.0
November 1998								
1007	59 a	78 a	17 a	27 c	10 c	6 cd	29.0 b	46.0 ab
2006	0 d	0	0	0	0 e	0 e	0.0 f	0.0
2008	0 d	0	0	0	0 e	0 e	0.0 f	0.0
2013	0 d	0	0	0	0 e	1 de	0.0 f	0.0
2015	0 d	0	0	0	0 e	0 e	0.0 f	0.0
2017	41 a	8 c	23 a	2	64 a	2 cde	43.0 a	5.0 c
2018	4 cd	0	1	0	48 b	0 e	18.0 c	0.0
3016	3 d	9 c	2 b	60 ab	2 de	26 b	2.0 ef	32.0 ab
3017	12 bc	33 b	2	37 bc	1 de	24 b	5.2 de	31.0 b
3020	15 b	11 c	9 a	97 a	6 cd	43 a	10.0 cd	50.0 a
May 1999								
2025	0 b	0 b	0	0	0 c	0 b	0.0 c	0.0 c
2028	0 b	0 b	0	0	0 c	0 b	0.0 c	0.0 c
2030	4 ab	6 a	3	4	8 ab	16 a	5.0 ab	9.0 a
2032	0 b	0 b	0	0	0 c	0 b	0.0 c	0.0 c
2033	0 b	0 b	0	0	0 c	0 b	0.0 c	0.0 c
2036	8 a	1 ab	3	1	5 bc	2 ab	5.2 ab	1.3 bc
2046	6 a	2 ab	13 a	5	19 a	1 b	13.0 a	3.0 ab
3021	4 ab	5 a	13 a	4	7 ab	4 ab	8.2 a	4.1 ab

[a]Isolate source: Kisoro = 1000 + , Kabale = 2000 + , Mbarara = 3000 + , Mbale = 4000 + .
[b]1724 is A1 tester isolate. For each seasons collection, within columns, means with the same letter(s) and no letters do not differ significantly at ($P \leqslant 0.05$) using Ls means PD, after square root transformation ($x + 0.5$).

Table 4
Oospore production by *P. infestans* from different parts of Uganda 1998–1999[a]

Production of oospores	Kisoro	Kabale	Mbarara	Mbale	Overall
No oospores	1 (0.3 $\pm$ 0.4)	10(3.3 $\pm$ 1.1)	3 (1.0 $\pm$ 0.6)	0 (0.0 $\pm$ 0.0)	14
Selfing only	2 (0.7 $\pm$ 0.5)	6 (2.0 $\pm$ 0.9)	0 (0.0 $\pm$ 0.0)	0 (0.0 $\pm$ 0.0)	8
Mating only	2 (0.7 $\pm$ 0.5)	8 (2.7 $\pm$ 1.0)	4 (1.3 $\pm$ 0.7)	4 (1.3 $\pm$ 0.7)	18
Both mating and selfing	3 (1.0 $\pm$ 0.6)	15(5.0 $\pm$ 1.3)	13(4.3 $\pm$ 1.3)	9 (3.0 $\pm$ 1.1)	40
Total	8	39	20	13	80

[a]Means in parenthesis indicate the average number of *P. infestans* isolates per season that produced oospores.

Table 5
Relationship between metalaxyl sensitivity and oospore production by *P. infestans* isolates from Uganda

Metalaxyl reaction	Total no of isolates	Non oospore producers	Oospore producing isolates in		
			Mating	Selfing	Mating and selfing
Resistant	36	3	11	2	20
Intermediates	19[a]	3	2	1	12
Sensitive	26	8	5	5	8
Total	81	14	18	8	40

[a]One isolate not tested for mating-type test. Resistant: growth on media with 5 and 100 μg/ml metalaxyl ⩾ 40% of 0 μg/ml; intermediates: growth on media with 5 μg/ml metalaxyl ⩾ 40% of 0 μg/ml; sensitive: growth on media with 5 and 100 μg/ml metalaxyl ⩽ 40% of 0 μg/ml.

Table 6
Pathogenicity of potato *P. infestans* on detached potato and tomato leaves

Isolate	Mean lesion length (mm) and percentage (%) area infected			
	Potato		Tomato	
	Lesion length	% area	Lesion length	% area
May 1998				
2002[a]	21.9 ± 2.4 (4.7)[b] b	40.4 ± 5.5 (40.1) b	19.3 ± 3.4 (4.2) ab	25.8 ± 5.7 (31.1) b
2003[a]	11.9 ± 2.9 (3.5) e	11.3 ± 3.1 (20.3) h	15.3 ± 3.0 (3.9) c	7.9 ± 1.5 (25.8) c
3008[a]	26.4 ± 4.8 (5.2) a	51.3 ± 13.2 (46.3) a	10.3 ± 1.7 (3.2) d	7.0 ± 2.4 (16.3) e
4001[a]	12.8 ± 2.2 (3.6) e	17.1 ± 3.7 (25.1) fg	11.1 ± 1.9 (3.4) d	16.2 ± 3.4 (24.4) cd
4002[a]	14.3 ± 2.4 (3.9) cd	20.0 ± 4.1 (27.2) ef	22.5 ± 3.0 (4.8) a	32.5 ± 2.9 (35.4) a
4003[a]	21.7 ± 2.1 (4.7) b	31.7 ± 4.7 (34.8) c	11.0 ± 0.6 (3.4) d	12.1 ± 2.1 (21.2) d
4004[a]	16.8 ± 2.4 (4.1) c	24.6 ± 3.7 (30.4) de	18.8 ± 2.2 (4.4) ab	26.7 ± 3.7 (31.7) b
4006[a]	22.2 ± 1.5 (4.8) b	26.3 ± 2.9 (31.4) cd	16.9 ± 1.7 (4.2) bc	20.0 ± 0.0 (27.3) c
1724[c]	11.8 ± 0.6 (3.5) e	13.3 ± 1.3 (22.2) gh	0.0 ± 0.0 (0.7)	0.0 ± 0.0 (5.7)
November 1998				
2009[a]	9.3 ± 1.0 (3.1) d	8.0 ± 1.8 (17.4) e	12.5 ± 3.1 (3.6) e	11.1 ± 2.9 (20.3) cd
2014[a]	22.9 ± 1.7 (4.8) b	16.9 ± 2.8 (25.0) c	15.8 ± 3.9 (4.0)cd	13.2 ± 3.5 (22.0) c
2015[a]	22.0 ± 4.8 (4.7) b	36.3 ± 11.0 (37.5) a	12.5 ± 1.7 (3.6) e	10.0 ± 4.1 (19.2 d
2016[a]	29.7 ± 2.1 (5.5) a	26.9 ± 3.7 (31.9) b	19.8 ± 1.3 (4.5) b	21.1 ± 2.8 (28.0) a
2018[a]	24.4 ± 2.4 (5.0) b	19.0 ± 1.9 (26.6) c	13.4 ± 1.3 (3.7) de	9.8 ± 2.2 (19.2) d
2021[a]	28.9 ± 4.2 (5.4) a	24.4 ± 1.6 (30.3) b	16.6 ± 2.4 (4.1) c	16.9 ± 0.8 (25.1) b
3015	15.5 ± 6.0 (3.9) c	15.0 ± 4.0 (23.5) d	0.0 ± 0.0 (0.7) f	0.0 ± 0.0 (5.7) e
3020[a]	24.7 ± 1.1 (5.0) b	19.9 ± 1.0 (27.2) c	23.5 ± 1.8 (1.8) a	20.7 ± 0.0 (27.7) ab
May 1999				
1009[a]	19.1 ± 1.4 (4.4) cd	18.8 ± 1.6 (26.4)	18.1 ± 3.4 (4.3) b	21.3 ± 2.1 (28.2)b
2025[a]	25.0 ± 1.6 (5.0) a	30.0 ± 2.8 (33.8) a	14.9 ± 1.9 (3.9) bcd	15.0 ± 2.0 (23.6)de
2027[a]	24.6 ± 1.8 (4.9) a	27.5 ± 2.0 (32.3) a	17.6 ± 0.6 (4.3) b	13.8 ± 1.4 (22.6)de
2030[a]	19.6 ± 1.9 (4.5) cd	19.6 ± 2.0 (27.0)	15.7 ± 1.4 (4.0) b	16.2 ± 3.4 (24.5)de
2034[a]	17.7 ± 0.5 (4.3) d	18.8 ± 1.6 (26.4)	17.3 ± 1.2 (4.2) b	18.3 ± 1.3 (26.1)bcd
2036[a]	20.5 ± 1.2 (4.5) b	22.1 ± 2.1 (28.7)	17.2 ± 1.6 (4.2) b	21.3 ± 4.0 (28.1) b
2038[a]	20.5 ± 2.1 (4.6) b	20.0 ± 4.1 (27.2)	12.5 ± 3.0 (3.6) d	12.5 ± 2.8 (21.5) e
2045[a]	21.9 ± 1.0 (4.8) ab	27.5 ± 5.2 (32.2) a	19.2 ± 3.2 (4.4) b	30.4 ± 7.5 (34.0) a
2046[a]	24.6 ± 1.8 (5.0) a	29.4 ± 5.5 (33.4) a	23.8 ± 1.0 (4.9) a	30.6 ± 5.1 (34.2) a
3021[a]	19.7 ± 1.2 (4.5) d	19.6 ± 0.9 (27.0)	13.8 ± 1.9 (3.8) c	15.2 ± 4.3 (23.6) de
4011[a]	17.4 ± 0.4 (4.3) d	17.9 ± 0.8 (25.8)	15.8 ± 2.3 (4.1) b	20.0 ± 3.3 (27.3) bc
4014[a]	20.1 ± 3.8 (4.5) b	20.0 ± 4.1 (27.2)	22.3 ± 5.6 (4.7) a	29.4 ± 3.7 (33.0) a

[a]*P. infestans* isolates that infected tomato detached leaves.
[b]Numbers in parenthesis indicate means of lesion length and percentage affected area after transformation. Pooled data for three experimental repeats.
[c]1724 is A1 isolate Kenya. For each seasons collection, within columns, means with the same letter(s) and no letters do not differ significantly at ($P \leqslant 0.05$) using Ls means PD, after square root transformation ($x + 0.5$) for lesion length and Arcsine transformation ($x + 1$) for percentage affected area.

Table 7
Relationship between metalaxyl sensitivity, oospore production and pathogenicity of *P. infestans* isolates on detached potato and tomato leaflets[a]

Metalaxyl reaction and oospore production	Total isolates tested	Number of isolates that infected	
		Potato	Tomato
Resistant			
No oospore producers	3	3	1
Selfing only	1	1	0
Mating only	9	9	2
Mating and selfing	14	13	10
Not tested for oospore	1	1	1
Total	28	27	14
Intermediates			
No oospore producers	2	2	1
Selfing only	1	1	1
Mating only	2	2	0
Mating and selfing	7	7	3
Total	12	12	5
Sensitive			
No oospore producers	6	6	1
Selfing only	4	4	1
Mating only	2	2	1
Mating and selfing	9	9	5
Total	21	21	8

[a]Resistant: growth on media with 5 and100 μg/ml metalaxyl ⩾ 40% of 0 μg/ml; intermediates: growth on media with 5 μg/ml metalaxyl ⩾ 40% of 0 μg/ml; sensitive: growth on media with 5 and 100 μg/ml metalaxyl ⩽ 40% of 0 μg/ml.

Ribeiro, 1996). Low metalaxyl dosage also tend to result in high frequencies of metalaxyl-resistant *P. infestans* isolates (Davidse et al., 1981; Dowley and O'Sollivan, 1981; Davidse et al., 1983) which might be the case in Uganda.

The high frequency of metalaxyl resistance in *P. infestans* isolates from Kabale points to the possibility that new biotypes of *P. infestans* may have emerged in Uganda. Highly resistant isolates were also found in samples from other parts of Uganda (Mbarara and Mbale), which are known to use little or no Ridomil to control late blight. In North Carolina, metalaxyl-resistant *P. infestans* isolates were recovered from potato fields to which no fungicides were applied (Fraser et al., 1999), but the greatest number of *P. infestans* isolates resistant to metalaxyl occurred in fields where metalaxyl was used.

The presence of relatively high numbers of metalaxyl-resistant *P. infestans* in Uganda is not necessarily a strong indicator of the presence of the A2 mating type. This is because there is no genetic correlation between resistance and mating type. It is also known that resistance to phenylamide became established in A1 populations before the appearance of the A2 type (Gisi and Cohen, 1996). Thus, the metalaxyl-resistant isolates in Uganda may still be of the A1 mating type. However, the high level of resistance to metalaxyl suggests that metalaxyl use should be planned carefully, as it could increase management costs (Bradshaw and Vaughan, 1996). Since Ridomil contains both Mancozeb and Metalaxyl, it is recommended to use Ridomil sparingly, and partially substitute it with Mancozeb alone or with other contact fungicides.

Some isolates produced oospores by selfing (10%), by both mating and selfing (50.0%), by mating alone (22.5%) and some produced no oospores (17.5%). The phenomenon of selfing is common in a number of *Phytophthora* species (Ko, 1988). Interestingly, of the 48-selfing isolates observed in the study, 40 also produced oospores by mating with the A1 tester strain. These results demonstrate selfing in some isolates and hybridization with A1 tester. This type of hybridization in the past suggested presence of A2 mating type (Hohl and Iselin, 1984). Since no A2 tester isolate was used in the tests, no definite conclusion can be made about the presence of A2 in Uganda. The results also show that oospores production by mating and selfing was not consistent. It is therefore not clear whether the oospores produced during mating are a result of hybridization between isolates (mating) or selfing. This can only be ascertained after *P. infestans* isolates from Uganda have been tested against both A1 and A2 tester isolates, and by isozyme analysis and DNA finger printing (Old et al., 1984; Goodwin, 1991). Nevertheless, the results point to the existence of varied populations of *P. infestans* in Uganda. Additionally, attempts should be made to examine whether oospores occur in nature in Uganda.

Results of metalaxyl resistance and mating tests revealed that the isolates that produced the largest number of oospores were those categorized as intermediary or highly resistant to metalaxyl. This is evidence of the possible existence of new *P. infestans* biotypes in Uganda. However, isolates 2021, 2028 and 2033 that were highly resistant to metalaxyl did not produce oospores in the mating tests. If these isolates belong to the A2 mating type, it would confirm the earlier finding that metalaxyl resistance was established in A1 populations before the appearance of A2 (Gisi and Cohen, 1996). Among the isolates studied, 69.2% of the metalaxyl-sensitive isolates produced oospores, but the numbers produced per plate were quite low (1–20 oospores/plate). The low numbers of oospores produced by the sensitive isolates indicate that these may be weak A2s in process of evolving to aggressive A2s, which probably have not yet developed resistance to metalaxyl or that they are actually A1s which produce oospores by selfing rather than mating. Metalaxyl has been reported to induce single-mating-type isolates of *P. infestans* to form oospores (Trout and Ristaino, 1999). The production of oospores in

P. infestans cultures from major potato growing districts of Uganda could be a result of continuous use of the fungicide by some potato farmers.

All *P. infestans* isolates examined in this study were pathogenic on potato, but only 44.2% were pathogenic on the detached tomato leaves. Furthermore, *P. infestans* isolates were more virulent on potato leaves than tomato. This finding reveals that the *P. infestans* isolates from potato were more aggressive on potato than tomato. Legard et al. (1995) also reported similar findings. However, its not known whether *P. infestans* from tomato are able to infect potato in the Ugandan situation, because there was no success in maintaining the tomato isolates for this test. In the USA, there are isolates which attack both tomato and potato (Goodwin et al., 1994; Goodwin et al., 1995).

The results of these experiments provide information essential for the development of effective disease control strategies. The presence of *P. infestans* isolates pathogenic on both potato and tomato requires that similar late blight management strategies be adopted for these crops. This is supported by the fact that over 50% of *P. infestans* isolates that caused disease on tomato leaves were highly resistant to metalaxyl, and the same isolates were able to produce oospores by mating and selfing. Thus, care must be taken to minimize use of metalaxyl (ridomil) on both crops. Certainly, to minimize further development of fungicide resistant strains, application of fungicides should be combined with the use of host resistance.

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