# Mating Type, Race Composition, Nuclear DNA Content, and Isozyme Analysis of Peruvian Isolates of *Phytophthora infestans*

P. W. Tooley, C. D. Therrien, and D. L. Ritch

First author: U.S. Department of Agriculture, Agricultural Research Service, Foreign Disease-Weed Science Research, Ft. Detrick Building 1301, Frederick, MD 21701; second and third authors: Department of Biology and Department of Plant Pathology, The Pennsylvania State University, University Park, 16802.

The authors thank Dr. V. Otazú, Hans Pinedo, and Dr. E. R. French, of the International Potato Center, Lima, Peru, for providing the isolates of *Phytophthora infestans* used in this study. The excellent technical assistance provided by Becky Huckle is also gratefully acknowledged.

Accepted for publication 28 November 1988 (submitted for electronic processing).

## ABSTRACT

Tooley, P. W., Therrien, C. D., and Ritch, D. L. 1989. Mating type, race composition, nuclear DNA content, and isozyme analysis of Peruvian isolates of *Phytophthora infestans*. Phytopathology 79:478-481.

Thirty-four isolates of *Phytophthora infestans* from potatoes growing in the Central Highlands of Peru were analyzed. All isolates were of the A1 mating type. Feulgen cytophotometry was used to measure the DNA content of individual zoospore nuclei. The distribution of DNA content in the Peruvian isolates was very similar to that previously observed in isolates from the United States and Europe. Races 0, 1, and 1,5 were the most common among the Peruvian isolates, representing 33, 39, and 15% of the population, respectively. The Peruvian population was monomorphic and identical to Mexican, U.S., and European populations at 11 enzyme loci. At two polymorphic loci, Peruvian isolates showed phenotypic distributions very similar to those of U.S. and European isolates. In terms of isozymes and races, fewer phenotypes were observed among Peruvian isolates than among isolates from Mexico, the United States, or Europe. These results strongly suggest a common ancestry for Peruvian, U.S., and European populations of *P. infestans*.

Late blight, caused by *Phytophthora infestans*, has been reported from virtually all major potato-growing regions of the world (4). Central Mexico is believed to represent the center of origin of the *P. infestans-Solanum* pathosystem (9,12). Here are found the largest concentrations of blight resistance genes (R genes), in wild *Solanum* species (7,9,10). In addition, the sexual stage of *P. infestans* was discovered in Central Mexico (5,6), where the two mating types (A1 and A2) of the fungus are found in approximately equal frequency.

Other characters also serve to distinguish Mexican isolates from those present in the United States and Europe. Mexican isolates contain greater numbers of virulence factors than isolates from other regions (19). In addition, ploidy differences have been observed between Mexican isolates and those from other regions (14,20).

*P. infestans* populations from areas other than Mexico, the United States, and Europe have rarely been studied. Although wild *Solanum* species abound in central Mexico, the Andes region of South America is considered to be the center of origin of the cultivated potato (4,9,13). Late blight occurs in the Andes region, and several wild sources of blight resistance have been found there (11). Bourke (1) suggested that the northern Andes region may have been the source of late blight that reached Europe and the United States in the 1840s, causing the Irish potato famine.

The recent occurrence of the A2 mating type in several European and Middle Eastern countries (8,15–17) has sparked renewed interest in the population biology of *P. infestans*. Characterization of Andean isolates of the fungus is desirable to determine whether they represent a population distinct from those present in Mexico, the United States, and Europe. Such information would be helpful in monitoring the global migration of *P. infestans* populations of both mating types.

The purpose of this study was thus to analyze *P. infestans* isolates from the Peruvian Andes, in terms of mating type, race composition, nuclear DNA content, and isozymes, and compare them with previously characterized isolates from Mexico, the United States, and Europe.



## MATERIALS AND METHODS

**Source of cultures.** Thirty-four *P. infestans* isolates from Peru were provided by Dr. V. Otazú and Dr. E. R. French, Pathology Department, International Potato Center, Lima, Peru. The isolates were collected from the foliage of blighted potatoes growing in the Central Highlands of Peru in 1984–1986 by Hans Pinedo, of the International Potato Center (Table 1).

**Mating type determination.** The mating type of each Peruvian isolate was determined by pairing it with isolates known to be of the A1 or the A2 mating type. Pairings were made on 20% V-8 juice agar in 9-cm-diameter plastic petri dishes at 18 C in darkness. A plug was cut with a number 2 cork borer from the colony margin of each Peruvian isolate. These plugs were paired with plugs from colonies of Mexican isolates 560 (A1 mating type) and 519 (A2 mating type), in separate petri dishes. After 3–4 wk, numerous oospores from one of the two pairings were visible in a distinct band where the two thalli coalesced. If oospores were formed in the A1 pairing, the unknown isolate was designated A2; if oospores were formed in the A2 pairing, the isolate was designated A1.

**Race identification.** Cultures of *P. infestans* were grown on Rye A agar (3) in 9-cm-diameter petri dishes for 10–14 days at 18 C in darkness. The cultures were washed with 10 ml of distilled water, and a cotton swab was used to prepare sporangial suspensions. The suspensions were concentrated by centrifugation at 1,000*g* in a tabletop centrifuge, and the concentration was adjusted to 50,000 sporangia per milliliter with a hemacytometer.

In vitro plantlets of 11 differential potato genotypes were obtained from the USDA Potato Introduction Station (Sturgeon Bay, WI). The genotypes and their major genes for late blight resistance (R genes derived from *S. demissum*) were as follows: the cultivar Kennebec (R1), PI 203905 (R2), PI 203902 (R3), PI 203900 (R4), PI 303146 (R5), PI 303148 (R7), PI 303149 (R8), Hodgson 2573 (R9), and PI 423656 (R10); the cultivars Norchip and Bintje, which contain no known R genes, were used as susceptible controls. The plantlets were propagated from cuttings, which were transplanted into pots of Jiffy-Mix Plus (Jiffy Products of America, West Chicago, IL) and grown in a growth chamber at 18–20 C. The chamber was illuminated with 40-W cool-white fluorescent tubes and incandescent bulbs (approximately 75 $\mu E \cdot m^{-2} \cdot sec^{-1}$) on a cycle of 16 hr light and 8 hr dark.

Leaf disks were cut from leaves of 4- to 8-wk-old plants, with a number 8 cork borer, and were placed lower surface upward on moist polyurethane foam in small, 16-compartment plastic boxes (Autogem, Ltd., Howley Park, Morley, Leeds, England). For each isolate to be tested, three leaf disks of each differential genotype were placed into one compartment within a box, so that each box contained three disks of each genotype (one box per isolate). A 40-$\mu$l drop of sporangial suspension of a given isolate was placed on the lower surface of each leaf disk in a single box. The boxes were covered with sliding plastic covers, placed in plastic bags to retain moisture, and incubated at 18 C in darkness.

The leaf disks were examined with a dissecting microscope for sporulation of *P. infestans* 7 days after inoculation. If sporulation was observed, the interaction was rated compatible; if no sporulation was observed, the interaction was rated incompatible. The number of compatible interactions for each isolate was recorded.

**Nuclear DNA content measurement.** Zoospores were produced as described by Caten and Jinks (3) and were fixed, postfixed, and stained. Their nuclear DNA content was determined by Feulgen cytophotometry, as described previously (20). Since the extinction coefficient for the Feulgen DNA complex was omitted from all calculations, DNA contents are reported only in arbitrary units (a.u.).

**Isozyme analysis.** Starch gel electrophoresis, staining procedures for specific enzymes, and allelic nomenclature were as previously described (18).

## RESULTS

All 34 Peruvian isolates were found to be of the A1 mating type.

TABLE 1. Accession numbers, sources, and pathogenic races of Peruvian isolates of *Phytophthora infestans*

| Isolate | Source in Peru[a] | Altitude (m) | Date collected | Race[b] |
|---|---|---|---|---|
| 800 | Comas, Junin | 2,800 | Feb. 1985 | 1 |
| 801 | Paucartambo, Cerro de Pasco | 2,500 | Feb. 1984 | 1 |
| 802 | Comas, Junin | 3,000 | Feb. 1985 | 0 |
| 803 | Paucartambo, Cerro de Pasco | 3,000 | Feb. 1984 | —[c] |
| 804 | Paucartambo, Cerro de Pasco | 3,500 | Feb. 1984 | 0 |
| 805 | Huasahuasi, Junin | 2,800 | Feb. 1984 | 0 |
| 806 | Paucartambo, Cerro de Pasco | 3,000 | Feb. 1984 | 1,5,10 |
| 807 | Comas, Junin | 3,000 | Feb. 1985 | 0 |
| 808 | Comas, Junin | 3,500 | July 1985 | 1 |
| 809 | Tarma, Junin | 3,000 | Feb. 1984 | 1 |
| 810 | Comas, Junin | 3,000 | Feb. 1985 | 1 |
| 811 | Acomayo, Huanuco | 2,000 | Feb. 1985 | 1,5,10 |
| 812 | Comas, Junin | 2,800 | Feb. 1985 | 1,5 |
| 813 | Comas, Junin | 2,000 | Feb. 1985 | 1,5 |
| 814 | Comas, Junin | 3,500 | Feb. 1985 | 1,5 |
| 815 | Comas, Junin | 2,000 | Nov. 1984 | 1,5,10 |
| 816 | Huasahuasi, Junin | 2,800 | Feb. 1985 | 0 |
| 817 | Comas, Junin | 2,500 | Feb. 1985 | 1,5 |
| 818 | Huancayo, Junin | 3,300 | Feb. 1985 | 1,5 |
| 819 | Huanuco, Huanuco | 2,000 | Feb. 1985 | 1,7 |
| 820 | Paucartambo, Cerro de Pasco | 3,000 | Feb. 1984 | 1 |
| 821 | Huasahuasi, Junin | 2,800 | Feb. 1984 | 0 |
| 822 | Ulcumayo, Junin | 3,500 | Feb. 1984 | 0 |
| 823 | Rosa Pampa, Huanuco | 2,000 | Feb. 1985 | 0 |
| 824 | Acomayo, Huanuco | 2,000 | Feb. 1985 | 1 |
| 825 | Atacocha, Cerro de Pasco | 2,800 | Feb. 1984 | 0 |
| 826 | Acobamba, Junin | 2,800 | Feb. 1984 | 0 |
| 827 | Huanuco, Huanuco | 2,000 | Feb. 1984 | 1 |
| 828 | Huanuco, Huanuco | 2,000 | Feb. 1985 | 1 |
| 829 | Huasahuasi, Junin | 2,800 | Feb. 1984 | 1 |
| 830 | Ulcumayo, Junin | 3,500 | Feb. 1984 | 0 |
| 831 | Huasahuasi, Junin | 2,800 | Nov. 1985 | 1 |
| 832 | Huanuco, Huanuco | 2,000 | Feb. 1986 | 1 |
| 833 | Huanuco, Huanuco | 2,000 | Feb. 1986 | 1 |

[a] Locality and department near which the isolate was collected.
[b] Numbers in this column, alone or separated by commas, indicate R-gene differentials that allowed infection and sporulation by *P. infestans*.
[c] Isolate 803 produced too few sporangia for race identification.

Races 0, 1, and 1,5 were the most common among the Peruvian isolates, representing 33, 39, and 15% of the population, respectively (Table 1).

Only 21 of the 34 Peruvian isolates produced sufficient numbers of zoospores to be tested for nuclear DNA content. Figure 1 is a histogram of the mean DNA content of all 21 Peruvian isolates tested. Two isolates (817 and 818) contained approximately one-half the DNA content of many other isolates and are probably diploid; the DNA contents of other isolates ranged from 0.77 to 1.22 a.u. (Table 2). Three isolates (806, 809, and 821) are presumed triploid, and the remaining 16 isolates appear to be tetraploid or of higher ploidy (Fig. 1 and Table 2). Figure 2 is histograms of the mean DNA content of specific isolates presumed to be diploid (isolate 818, 0.53 a.u.), triploid (isolate 806, 0.82 a.u.), and tetraploid (isolate 816, 1.06 a.u.).

Isozyme phenotypes observed in the Peruvian isolates were identical to those previously observed in Mexican, U.S., and European populations of *P. infestans* (18). The Peruvian population was monomorphic and identical to Mexican, U.S., and European populations at 11 enzyme loci (*Dia-2, Gk-1, Gpi-2, B-Glu-2, Gr-2, Ldh, Mdh-2, Madh, Pgd, Sod-1*, and *Sod-2*). The Peruvian population was also monomorphic at two enzyme loci (*Me* and *Xdh*) at which U.S. and European populations were monomorphic but at which the Mexican population was polymorphic (18). Finally, the Peruvian population was polymorphic at two loci (*Gpi-1* and *Pep*) at which Mexican as well as U.S. and European populations were polymorphic (18). However, at these two loci, slightly fewer phenotypes were observed among the Peruvian isolates than had been observed among Mexican, U.S., and European isolates (Table 3).

## DISCUSSION

In all traits examined, Peruvian isolates of *P. infestans* were very similar to U.S. and European isolates, strongly suggesting a common ancestry for Peruvian, U.S., and European populations of this fungus.

All the Peruvian isolates were of the A1 mating type, which was the only mating type found in the United States and Europe until recently (8,15–17). The source of the isolates of the A2 mating type recently introduced into Europe remains unknown, but these results indicate that they probably did not originate in Peru or the Andes region.

Fewer pathogenic races were observed among Peruvian isolates of *P. infestans* than were previously observed among isolates from the United States and Europe (19). However, race 0 was the most common in both groups of isolates, and both groups showed lower virulence than Mexican isolates (19). The low number of pathogenic races in Peru may result from the fact that cultivars containing major genes originating from *S. demissum* have only been grown there for about the past 30 years. The relatively high frequency of race 1 in Peru may reflect the increased planting of

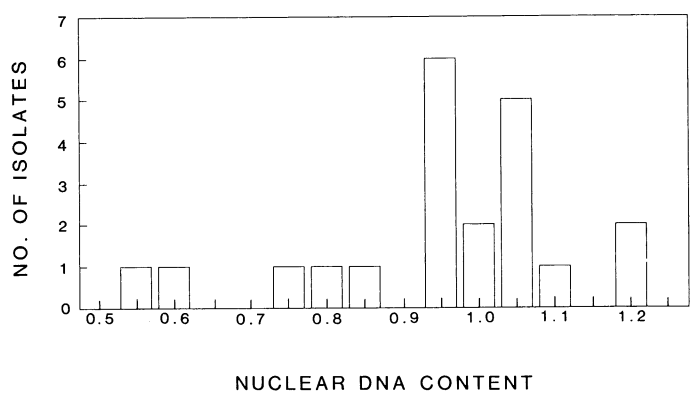


**Fig. 1.** Histogram of mean nuclear DNA content, as determined by Feulgen cytophotometry (in arbitrary units), of zoospores of 21 Peruvian isolates of *Phytophthora infestans*.

TABLE 2. Mean nuclear DNA contents of 21 Peruvian isolates of *Phytophthora infestans*, as determined by Feulgen cytophotometry

| Isolate | Mean DNA[a] | SE[b] | Isolate | Mean DNA | SE |
|---|---|---|---|---|---|
| 800 | 1.18 | 0.03 | 820 | 1.05 | 0.04 |
| 801 | 1.06 | 0.04 | 821 | 0.77 | 0.03 |
| 802 | 0.96 | 0.03 | 822 | 1.06 | 0.04 |
| 806 | 0.82 | 0.03 | 826 | 1.22 | 0.04 |
| 807 | 0.98 | 0.04 | 827 | 1.02 | 0.04 |
| 808 | 1.09 | 0.03 | 828 | 0.96 | 0.05 |
| 809 | 0.84 | 0.03 | 830 | 1.02 | 0.04 |
| 816 | 1.06 | 0.02 | 831 | 0.94 | 0.03 |
| 817 | 0.61 | 0.02 | 832 | 1.03 | 0.03 |
| 818 | 0.53 | 0.02 | 833 | 0.96 | 0.03 |
| 819 | 0.94 | 0.04 | | | |

[a] Data are means from 50 nuclei per isolate and are expressed in arbitrary units.
[b] Standard error of the mean.

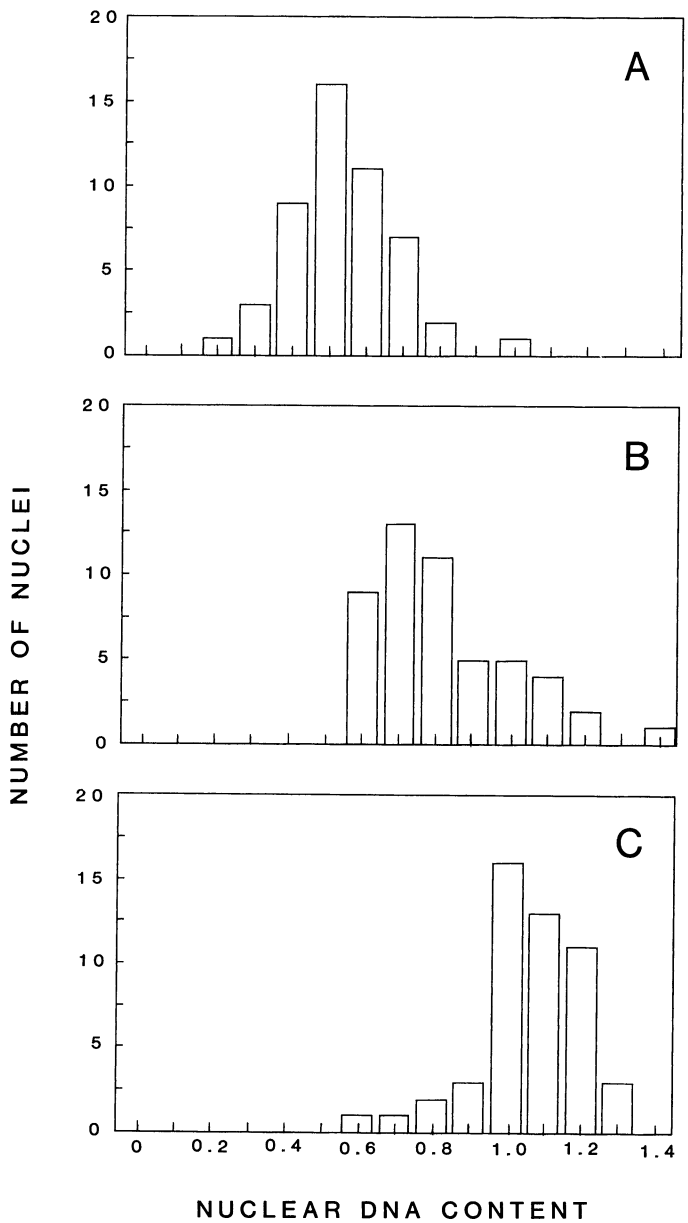


**Fig. 2.** Histograms of nuclear DNA content, as determined by Feulgen cytophotometry (in arbitrary units, a.u.), of zoospores of three Peruvian isolates of *Phytophthora infestans*; 50 nuclei were measured per isolate. Isolate number, mean nuclear Feulgen DNA content, and presumed ploidy are as follows: **A,** isolate 818, 0.53 a.u., diploid; **B,** isolate 806, 0.82 a.u., triploid; **C,** isolate 816, 1.06 a.u., tetraploid.

cultivars containing R1 there, compared with the United States and Europe. Several popular cultivars now grown in the Central Highlands of Peru, such as Mariva and Revolucion, contain resistance gene R1.

The DNA content of Peruvian isolates of *P. infestans* ranged from 0.53 to 1.22 a.u. (mean value 0.95 a.u.), and the Peruvian population appeared to contain diploid, triploid, and tetraploid isolates. These values and ploidy levels are nearly identical to those observed previously in a group of U.S. and European isolates (20). Mexican isolates, which cytological studies indicated were diploid (2,14), had DNA contents ranging from 0.48 to 0.79 a.u. (mean value 0.59 a.u.) (20). The skewness in the histograms of DNA content may be due to DNA replication in zoospores during mitotic interphase or to the presence of some nuclei of higher ploidy in the population. Future studies involving larger numbers of isolates of both mating types should attempt to determine the relationship between mating type and ploidy levels in *P. infestans* populations (17).

Isozyme analysis revealed that at 11 enzyme loci the Peruvian population of *P. infestans* was monomorphic and identical to populations from Mexico, the United States, and Europe. However, at two loci at which polymorphism was observed in other *P. infestans* populations (18), Peruvian isolates showed phenotypic distributions almost identical to those of U.S. and European isolates. As with pathogenic races, fewer isozyme phenotypes were observed at these loci in Peruvian isolates than in isolates from Mexico, the United States, and Europe.

Our results provide additional evidence to support the hypothesis (9,12) that central Mexico, and not the Andes region, is the center of origin of the *P. infestans-Solanum* pathosystem. If the Andes region were a separate center of origin of *P. infestans*, one would expect to find both mating types and higher levels of genetic variation than we observed, in characters such as pathogenic races and isozymes.

The results of our studies are consistent with the nineteenth-century theory (1) that late blight could have been introduced into Europe from the northern Andes region of South America. Bourke (1) believed that *P. infestans* probably existed in the northern Andes prior to 1845, since genes for hypersensitivity to blight occur there in wild potatoes (7,11). Furthermore, the increased trade between the newly independent South American states, Europe, and North America occurring around 1840 provided a strong mechanism for the potential introduction of *P. infestans* into Europe (1). It is clear that potatoes were shipped from the northern Andes to Europe during this time, as some newly introduced South American potato varieties were among those that succumbed to late blight in Belgium in 1845 (1).

Additional data are required, however, before firm conclusions can be drawn regarding the source of European and U.S. isolates of

TABLE 3. Genotypes observed at the *Gpi-1* and *Pep* loci in Mexican, U.S., European, and Peruvian isolates of *Phytophthora infestans*

| | Number of individuals in population[a] | | |
|---|---|---|---|
| | Mexican isolates | U.S. and European isolates | Peruvian isolates |
| *Gpi-1* genotype[b] | | | |
| 83/100 | 1 | 0 | 0 |
| 86/86 | 1 | 0 | 0 |
| 86/100 | 6 | 41 | 34 |
| 100/100 | 36 | 5 | 0 |
| 100/122 | 5 | 0 | 0 |
| 122/122 | 1 | 0 | 0 |
| *Pep* genotype[b] | | | |
| 92/92 | 3 | 1 | 0 |
| 92/100 | 15 | 42 | 33 |
| 100/100 | 32 | 3 | 1 |

[a] Data for Mexican, U.S., and European isolates were obtained previously (18) and are presented for purposes of comparison.
[b] Genotypes are designated according to relative electrophoretic mobility, as described previously (18).

*P. infestans*. Other regions of the world with similar isolates might also be considered possible sources of U.S. and European strains. Thus, further studies analyzing isolates from other regions are desirable to help elucidate the genetic structure and patterns of migration of *P. infestans* populations.

## LITERATURE CITED


1. Bourke, P. M. A. 1964. Emergence of potato blight, 1843–46. Nature 203:805-808.
2. Brasier, C. M., and Sansome, E. 1975. Diploidy and gametangial meiosis in *Phytophthora cinnamomi, P. infestans*, and *P. drechsleri*. Trans. Br. Mycol. Soc. 65:49-65.
3. Caten, C. C., and Jinks, J. L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Can. J. Bot. 46:329-348.
4. Cox, A. E.,. and Large, E. C. 1960. Potato blight epidemics throughout the world. U.S. Dep. Agric., Agric. Handb. 174. 230 pp.
5. Galindo, J., and Gallegly, M. E. 1960. The nature of sexuality in *Phytophthora infestans*. Phytopathology 50:123-128.
6. Gallegly, M. E., and Galindo, J. 1958. Mating types and oospores of *Phytophthora infestans* in nature in Mexico. Phytopathology 48:274-277.
7. Hawkes, J. G. 1958. Significance of wild species and primitive forms for potato breeding. Euphytica 7:257-270.
8. Hohl, H. R., and Iselin, K. 1984. Strains of *Phytophthora infestans* with A2 mating type behaviour. Trans. Br. Mycol. Soc. 83:529-531.
9. Niederhauser, J. S., and Cobb, W. C. 1959. The late blight of potatoes. Sci. Am. 200:100-112.
10. Niederhauser, J. S., and Mills, W. R. 1953. Resistance of *Solanum* species to *Phytophthora infestans* in Mexico. Phytopathology 43:456-457.
11. Ochoa, C. 1954. Northern Peru, a possible new source of potatoes resistant to *Phytophthora infestans*. Am. Potato J. 31:371.
12. Reddick, D. 1939. Whence came *Phytophthora infestans*? Chron. Bot. V 4/6:410-412.
13. Salaman, R. N. 1952. The social influence of the potato. Sci. Am. 187:50-52, 54, 56.
14. Sansome, E. 1977. Polyploidy and induced gametangial formation in British isolates of *Phytophthora infestans*. J. Gen. Microbiol. 99:311-316.
15. Shaw, D. S., Fyfe, A. M., Hibberd, P. G., and Abdel-Sattar, M. A. 1985. Occurrence of the rare A2 mating type of *Phytophthora infestans* on imported Egyptian potatoes and the production of sexual progeny with A1 types from the U.K. Plant Pathol. 34:552-556.
16. Tantius, P. H., Fyfe, A. M., Shaw, D. S., and Shattock, R. C. 1986. Occurrence of the A2 mating type and self-fertile isolates of *Phytophthora infestans* in England and Wales. Plant Pathol. 35:578-581.
17. Therrien, C. D., Ritch, D. L., and Christ, B. J. 1987. Cytophotometric analysis of the nuclear DNA content of 23 isolates of *Phytophthora infestans* from the Netherlands. (Abstr.) Phytopathology 77:1620.
18. Tooley, P. W., Fry, W. E., and Villarreal Gonzalez, M. J. 1985. Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. J. Hered. 76:431-435.
19. Tooley, P. W., Sweigard, J. A., and Fry, W. E. 1986. Fitness and virulence of *Phytophthora infestans* isolates from sexual and asexual populations. Phytopathology 76:1209-1212.
20. Tooley, P. W., and Therrien, C. D. 1987. Cytophotometric determination of the nuclear DNA content of 23 Mexican and 18 non-Mexican isolates of *Phytophthora infestans*. Exp. Mycol. 11:19-26.