Characteristics of *Phytophthora infestans* Isolates and Development of Late Blight on Tomato in Taiwan

G. L. Hartman, USDA/ARS, University of Illinois, Urbana 61801-4709; **Y. H. Huang**, AVRDC, Shanhua, Tainan, Taiwan, ROC

ABSTRACT

Hartman, G. L., and Huang, Y. H. 1995. Characteristics of *Phytophthora infestans* isolates and development of late blight on tomato in Taiwan. Plant Dis. 79:849-852.

Thirteen isolates of *Phytophthora infestans* were grown on various solidified media. Growth of all isolates was best on rye A agar. Mean sporangia length and width ranged from 23.5 to 41.7 μm × 13.9 to 23.4 μm, respectively, with a length/width ratio of 1.69 to 1.92. All isolates were A1 mating type and all isolates, except one isolate from potato, were classified race T1 because blight developed on three tomato lines with the *Ph1* resistance gene. Area under the disease progress curve (AUDPC) differed significantly ($P < 0.05$) by isolate on both detached tomato leaflets and pot-cultured seedlings. Isolate Pi11 from potato had the lowest AUDPC values on detached leaves and seedlings. Late blight occurred on tomato seedlings transplanted monthly in the central highlands of Taiwan with greatest disease severity from March to June. Field-grown tomatoes sprayed with metalaxyl once and twice per week reduced late blight AUDPC values, decreased yield losses, and increased fruit numbers, compared with the results from fewer or no sprays. Disease, measured as AUDPC, correlated negatively ($P < 0.05$) to yield ($r = -0.82$) and number of fruit ($r = -0.76$).

Tomatoes (*Lycopersicon esculentum* Mill.) are grown throughout the world and are important commercially for fresh market and processing. Diseases are often a factor that limits production. One disease, late blight caused by *Phytophthora infestans* (Mont.) de Bary, occurs in temperate and highland tropical regions on potato and tomato (7). Late blight is one of the most destructive diseases of tomato because the fungus attacks foliage and stems, often killing plants and rotting fruits. The fungus can be difficult to culture, although several reports indicate suitable cultural media for growth (2,11,18).

In Taiwan, late blight was first reported in 1919 (14), and a report in 1990 confirmed that *P. infestans* occurred on herbarium specimens collected by Sawada (9). Even though the disease normally occurs during the summer in the highlands and during the winter in the lowlands of Taiwan on potatoes and/or tomatoes, the first report of culturing *P. infestans* in Taiwan was in 1991 (8).

Phytophthora infestans has two mating types (5). It was not until 1984 that the A2 mating type was isolated outside of Mexico (10). Since then, it has been found in Europe, North America, and Asia (17). In addition to mating types, the fungus can be characterized by its two races, T0 and T1, which are distinguished on tomatoes carrying the *Ph1* resistance gene (6). Commercial varieties carrying resistance to race T1 are not available and metalaxyl has been applied to control epidemics of late blight (1). Greenhouse and field isolates with resistance to metalaxyl have been reported (4,12). The objectives of our study were to (i) characterize *P. infestans* isolates from Taiwan by comparing growth rates on different media, sporangia, mating types, races, and virulence, (ii) monitor disease development in different environments, and (iii) evaluate metalaxyl for control of tomato late blight.

Corresponding author: G. L. Hartman;
E-mail: hartman@ux1.cso.uiuc.edu

Mention of a trade name of proprietary product does not constitute a guarantee or warrantee by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Accepted for publication 4 May 1995.

MATERIALS AND METHODS

Isolation, maintenance, and inoculum. Field-infected tomato and potato leaves collected from three regions in Taiwan were sectioned into 4-mm^2 pieces, soaked in 1% sodium hypochlorite for 3 min, rinsed in sterile distilled water for 3 min, and plated on rye A agar (2). After 5 to 7 days at 18°C, hyphal tips were transferred to rye A agar. For comparison, a second isolation method was used to wash diseased leaves. These were incubated at 18°C for 5 days on moist filter paper in 15-cm culture dishes, and sporangia were collected from leaves by rinsing with sterile distilled water. One milliliter of the sporangial suspensions was placed on rye A agar in 15-cm culture dishes. Hyphal tips from 3-day-old colonies were transferred to rye A agar. In total, 13 isolates were obtained and cultured.

To produce inoculum, cultures grown on rye A agar were flooded with 10 ml of sterile distilled water and sporangial suspensions were sprayed on detached tomato leaves of line L3975 (var. TK 70, Asian Vegetable Research and Development Center [AVRDC], Shanhua, Taiwan) and incubated for 14 days under 10 h of light (68 μE m^{-2} s^{-1}) per day in 15-mm culture dishes. Leaves were washed and the suspension was used to inoculate plants. Cultures were stored in 2-ml vials with 15% dimethyl sulfoxide (19) at –80°C for long-term storage.

Characteristics of isolates. Thirteen isolates were cultivated on corn meal, lima bean, oat meal, pea meal, pea seeds, rye A, V8 juice, and clarified V8 juice agars (3). Two-millimeter-diameter agar plugs from 14-day-old cultures grown on rye A agar from each of the 13 isolates were transferred to the center of each media and incubated at 18°C in darkness. After 10 days the colony diameters were measured.

Sporangial suspensions from the 13 isolates were obtained by washing inoculated detached leaves of L3975 with sterile distilled water. A drop of suspension was placed on a microscope slide and the length and width of 100 sporangia were measured with a micrometer under a bright-field compound microscope at 400× magnification. The length/width (LW) ratio was calculated for each sporangium measured.

To test mating types, 4-mm^2 agar plugs from the edge of 2-week-old cultures of each isolate were transferred about 2-cm off center in a rye A agar plate. Known mating types, A1 type (Ca65) or A2 type (E13a), of *P. infestans* (16) were placed about 2 cm off center on the other half of the culture dish. Cultures were incubated in the dark at 18°C. The occurrence of oospores was determined using a bright-field compound microscope 10 to 14 days after isolate pairing.

Thirty-day-old seedlings of AVRDC tomato lines L1497 (Pi204976), L1501 (Pi204980), and L1517 (Pi 204996) (all with the *Ph1* gene for resistance), and L3975 (with no known genes for resistance), were inoculated by atomizing with a sporangial suspension adjusted to concentrations of 10^3 to 10^4 sporangia per ml. Five replications of two plant samples of each line were inoculated with each isolate using a randomized complete block design. Immediately after inoculation, the seedlings were placed in a growth room

with overhead sprinklers that misted water on the foliage for 20 s every 20 min under 14 h of light (49 μE m^{-2} s^{-1}) per day at 22±2°C. Disease severity was rated from 8 to 12 days after inoculation using the following 1 to 6 assessment scale: 0 = no symptoms; 1 = 1 to 10% of leaf area affected (LAA); 2 = 11 to 20% LAA; 3 = 21 to 40% LAA; 4 = 41 to 70% LAA; 5 = 71 to 90% LAA; and 6 = 91 to 100% LAA or plant dead.

Virulence of isolates. Tomato leaflets were removed from 30-day-old greenhouse-grown plants of L3975, placed in 9-cm-diameter culture dishes on moist filter paper, and inoculated individually with 0.02-ml drops of an adjusted spore concentration (10^4 sporangia per ml) of each isolate on either side of the midrib. After inoculation, leaflets were incubated under 12 h of light (68 μE m^{-2} s^{-1}) per day at 18°C. Each isolate was replicated four times; each replicate consisted of four leaflets. Disease severity was rated daily using the following 1 to 6 assessment scale: 0 = no reaction; 1 = restricted lesions; 2 = expanded lesions but little or no mycelia and sporangia production on inoculated sites; 3 = expanded lesions with mycelia and sporangia production on inoculated sites; 4 = expanded lesions with mycelia and sporangia covering 10 to 50% of the leaflet surface; 5 = expanded lesions with mycelia and sporangia covering 51 to 90% of the leaflet surface; and 6 = expanded lesions with mycelia and sporangia covering 91 to 100% of the leaflet surface.

Thirty-day-old seedlings of tomato line L3975 sown in 9-cm-diameter pots were inoculated with 1×10^4 sporangia per ml of individual isolates and incubated in a growth room under conditions described previously. There were four replications for each isolate and three plants for each replication. Disease severity of inoculated plants was rated daily after inoculation using the scale described in the previous section. Area under the disease progress curve (AUDPC) was calculated (15) and values were used in analysis of variance (ANOVA) with means separated by least significant difference (LSD) ($P < 0.05$).

Late blight on field-grown tomatoes. From August 1991 to June 1993, 30-day-old seedlings of a commercial variety, Taichung ASVEG #4 (TA4) and L1197 (AVRDC germ plasm), were transplanted at monthly intervals to 19-cm-diameter clay pots (one plant per pot) and moved to the field at the Puli Branch Station, Taichung District Agricultural Improvement Station (Taichung DAIS) in Taichung, Taiwan. Plants were arranged in four rows with 0.75 m between rows and 0.5 m between plants in a row. Disease severity based on percentage of leaf area affected (described previously) was rated 30 days after transplanting. In addition, temperature, humidity, and precipitation were recorded daily.

Effects of metalaxyl. A metalaxyl (Ridomil MZ58, Ciba Crop Protection, CIBA-GEIGY Corp., Summit, N.J.) stock solution, containing 0.1 g of metalaxyl and 0.5 ml of dimethyl sulfoxide per 1 liter of sterile distilled water, was added to 99 ml of molten sterile rye A agar yielding 10 μg of metalaxyl per ml. Two-millimeter-diameter disks from the margin of 10-day-old colonies of each *P. infestans* isolate grown on rye A agar were placed on the center of unamended and metalaxyl-amended rye A agar. Isolates were classified as sensitive, intermediate, and resistant isolates to metalaxyl as described by Shattock (16).

Thirty-day-old tomato seedlings of TA4 and L3975 were transplanted in 20-cm-diameter clay pots and moved to the field at Puli Branch Station, Taichung DAIS. There was a space of 0.75 m between rows and 0.5 m between pots in a row. Metalaxyl was applied with a power backpack sprayer at a concentration of 2.2 kg per ha at a frequency of either twice or once weekly; once every 2 weeks; or once every 4 weeks. Control plants were not sprayed with fungicide. Treatments and lines were arranged as a factorial in a randomized complete design using four replications and five plants for each replication. Disease severity, based on the 1 to 6 rating scale described previously, was rated at 10-day intervals after transplanting. Weights and fruit numbers of freshly harvested red-ripe fruits from several harvests were combined. AUDPC values were analyzed using ANOVA and means were separated by LSD ($P < 0.05$). The experiment was conducted from October 1991 to January 1992. The experiment was repeated from July to October 1992; however, data were not included as there was abundant rainfall including a typhoon early in the experiment that reduced some experimental units, and very little rainfall later, which drastically reduced disease severity levels.

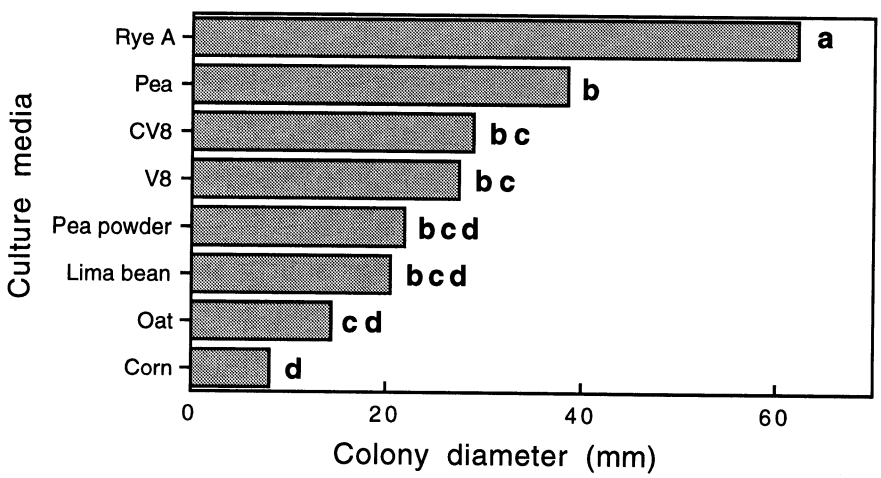

Fig. 1. Mean radial diameter of thirteen isolates of *Phytophthora infestans* on rye A, pea seed, clarified V8 juice (CV8), V8 juice (V8), pea meal, lima bean, oat meal, and corn meal media. Same letter beside bars indicates means were not different significantly according to least significant difference ($P < 0.05$).

RESULTS

Characteristics of isolates. All isolates grew on all media, but colony diameter was significantly ($P < 0.05$) larger on rye A medium (Fig. 1). Sporangia of all isolates were ellipsoid to ovoid and semi-papillate. The sporangial length and width of the tomato isolates ranged from 36.2 to 41.7 μm and from 19.3 to 23.4 μm, respectively. The mean length and width of sporangia from the potato isolate Pi11 were 23.5 μm and 13.9 μm, respectively. Sporangia LW ratios ranged from 1.69 to 1.92. Pedicels were short or absent and could not be measured accurately. All isolates were identified as A1 mating type based on paired cultures with known races. All isolates, except Pi11, caused symptoms on plants with and without the *Ph1* gene for resistance. Severity ratings ranged from 0.9 to 6.0. Mean severity ratings over isolates were 0.8, 0.6, 0.3, and 2.1, respectively for L1497, L1501, L1517, and L3975. Lesions did not expand on lines with the *Ph1* gene for resistance but lesions did expand on line L3975, which has no known genes for resistance.

Virulence of isolates. Lesions developed on detached tomato leaflets and tomato foliage 3 days after inoculation. Seven days after detachment, some isolates caused blight on >90% of the foliage. AUDPC values differed significantly ($P < 0.05$) by isolate. Isolates Pi6, Pi10, and Pi12 caused consistently greater disease than most other isolates, reflected by higher AUDPC values on both detached leaflets and on inoculated foliage (Table 1). Pi11 had lower AUDPC values than those of other isolates on detached leaves and seedlings.

Late blight on field-grown tomato. Late blight developed each month on tomato seedlings at the Puli location with greatest disease severity from March to

June, a time when moderate rainfall and temperature, and high humidity levels, prevailed. Monthly maximum and minimum mean temperatures ranged from 18.6 to 29.7°C and 7.6 to 20.4°C, respectively, at the Puli Branch Station during the period from August 1991 to June 1993 (Fig. 2). Mean monthly humidity ranged from 59.1 to 94.0%. Disease levels peaked after the maximum and minimum temperatures were at their lowest. Leaf lesions initially were brownish-green and 1 to 2 mm in diameter. Lesions enlarged to form irregularly water-soaked, dark green necrotic areas that coalesced. Stem lesions were irregular in shape and blackish-gray. Fruit lesions were round to irregular and brown to brownish-black.

Effects of metalaxyl. None of the isolates grew well on rye A medium containing 10 µg metalaxyl per ml, compared with nonamended medium. Late blight development in the field on TA4 and L1197 sprayed four to eight times per month with metalaxyl was slower than when metalaxyl was sprayed less frequently or not at all (Fig. 3). For each treatment, L1197 had lower AUDPC values than TA4 (Table 2). AUDPC values for both tomato lines were significantly ($P < 0.05$) higher when metalaxyl was applied once every 2 weeks or not at all. There were no significant ($P < 0.05$) differences in the AUDPC values between plots receiving two and one sprays per week within each line. As the AUDPC values increased, yields and fruit numbers decreased in both lines. As AUDPC increased, fruit yield ($r = -0.82$) and fruit numbers ($r = -0.76$) decreased significantly ($P < 0.05$). Yield and fruit numbers from plants in plots with one- or two-per-week fungicide applications within each tomato line did not differ significantly ($P < 0.05$), but were significantly ($P < 0.05$) less when fungicide was applied less than once per week, except for the number of fruits in L1197.

DISCUSSION

The results of our initial attempts to culture *P. infestans* were somewhat inconsistent and limited in success. Once cultured, the fungus grew poorly on most media except for rye A agar. In Taiwan, rye seed is difficult to obtain compared with ingredients of other media tested. This problem was solved when rye seed was imported. Morphologically, our isolates had sporangia and an LW ratio within the described range of *P. infestans* (22), although the potato isolate had sporangia smaller than those of the tomato isolates. This potato isolate could have been different due to its association with potatoes

Table 1. Area under the disease progress curve (AUDPC) values for late blight on detached tomato leaflets and tomato foliage of L3975 inoculated with thirteen isolates of *Phytophthora infestans*

	AUDPC[a]	
Isolate	Detached leaflet	Seedlings
Pi1	6.0	10.9
Pi2	7.4	11.6
Pi3	9.4	7.5
Pi4	8.3	12.9
Pi5	10.3	13.9
Pi6	10.3	17.1
Pi7	7.1	18.5
Pi8	2.9	18.3
Pi9	6.4	21.6
Pi10	10.0	20.1
Pi11	1.4	5.4
Pi12	9.9	19.4
Pi13	5.6	18.4
LSD[b] ($P < 0.05$)	1.5	4.1

[a] Calculated based on a formula from Shaner and Finney (15) using the following severity assessment scale: 0 = no symptoms; 1 = 1 to 10% of leaf area affected (LAA); 2 = 11 to 20% LAA; 3 = 21 to 40% LAA; 4 = 41 to 70% LAA; 5 = 71 to 90% LAA; and 6 = 91 to 100% LAA or plant dead. Means were based on four replicaitons and three samples each.

[b] Least significant difference.

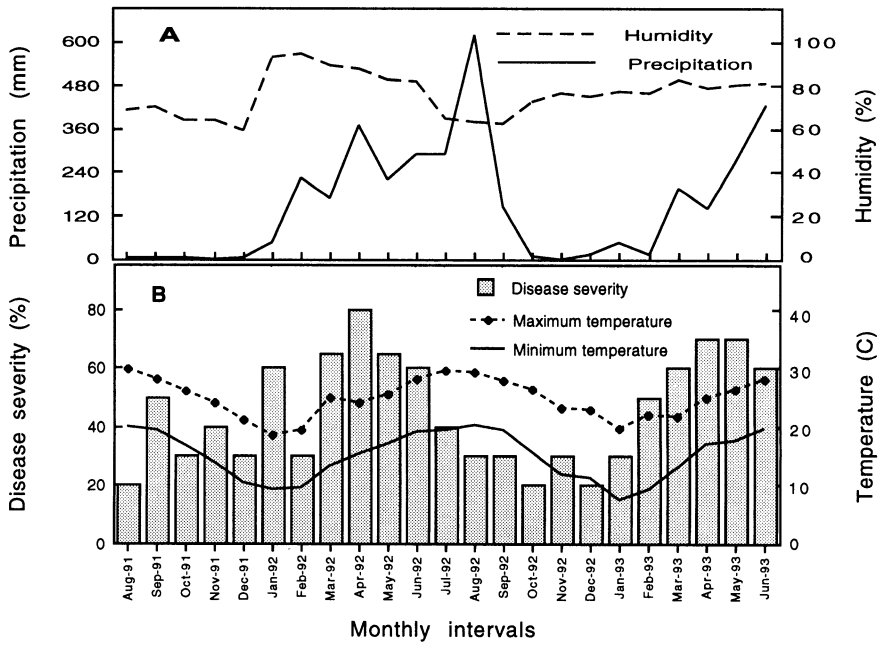

Fig. 2. Late blight severity 30 days after transplanting 30-day-old tomato seedlings at Puli Branch Station, Taichung District Agriculture Improvement Station. Monthly means of **(A)** humidity and precipitation and **(B)** temperature were recorded from August 1991 to June 1993.

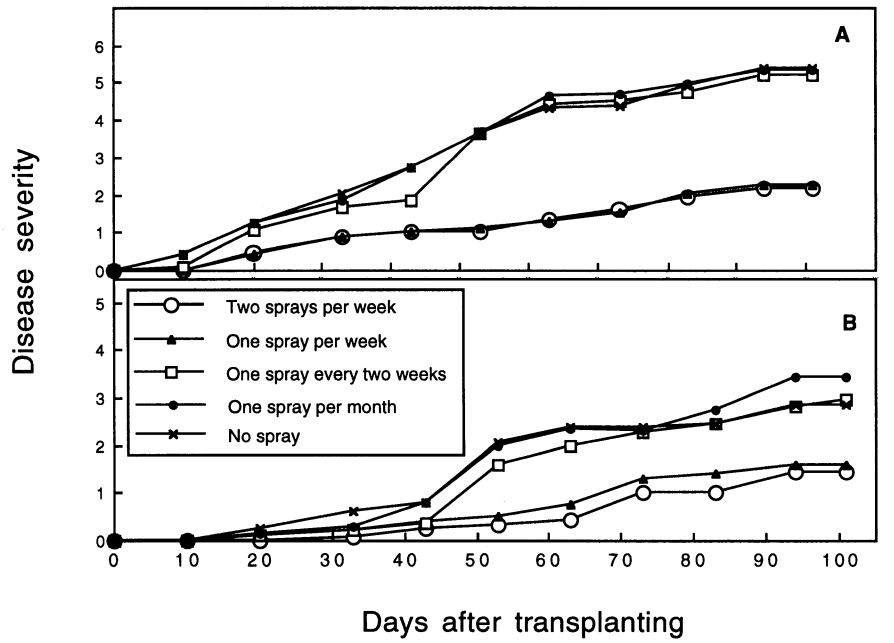

Fig. 3. Development of late blight on **(A)** tomato var. Taichung ASVEG No. 4 and **(B)** AVRDC accession L1197 that were either sprayed or not sprayed with metalaxyl at various intervals.

Table 2. Area under the disease progress curve (AUDPC) for late blight and yield of two tomato lines TA4 and L1197 that were either sprayed or not sprayed with metalaxyl at various intervals during the growing season

Metalaxyl applications	AUDPC[a]	Yield (ton per ha)	Yield loss (%)	Fruit no. (× 10^5 per ha)	Fruit no. loss (%)
TA4[b]					
Two per week	113	100.0	...	7.8	3.7
One per week	115	91.3	8.7	8.1	...
One every 2 weeks	291	53.9	46.1	3.9	51.9
One per month	316	50.8	49.2	4.8	40.7
None	309	41.0	59.0	3.7	54.3
LSD[c] ($P < 0.05$)	24	28.5		2.3	
L1197[d]					
Two per week	50	100.0	...	23.2	8.3
One per week	68	109.8	...	25.3	...
One every 2 weeks	129	75.9	30.9	18.8	25.7
One per month	152	80.0	27.1	19.3	23.7
None	148	80.7	26.5	18.9	25.3
LSD ($P < 0.05$)	28	17.5		5.2	

[a] Calculated based on a formula from Shaner and Finney (15) using a 0 to 6 scale from four replications.
[b] Taichung ASVEG No. 4.
[c] Least significant difference.
[d] AVRDC germ plasm.

possibly over many generations. Alternatively, it could represent a degenerative type because it also exhibited poor colonization of tomato leaves. All the isolates we tested were A1 mating types and they probably represent "old populations" (17) imported initially on seed potato. All tomato isolates were race T1, which are reported to be more prevalent and virulent than TO (21).

Isolates in our study varied in their virulence, based on AUDPC values of results from inoculated seedlings or detached leaves. Because of this inconsistency, it may not be useful to evaluate isolate virulence and host resistance or response under in vitro conditions. However, this method may give some indication of reproduction capacity, as was shown for virulence of potato isolates of *P. infestans* based on the production of sporulating lesions on detached leaflets (20).

Tomatoes transplanted monthly in the central highlands of Taiwan developed late blight throughout the year, but the disease was especially severe from March through June, which corresponds to the season of tomato production in the highlands. Disease severity was much lower between August and December when precipitation was less. At lower elevations in Taiwan, tomato production occurs in the winter and early spring when temperatures and humidity are extremely favorable for the pathogen. Thus, tomato production in Taiwan takes place during periods when late blight is most likely to occur and commercially grown varieties are susceptible to the races that are present.

The Taiwanese isolates of *P. infestans* we tested were sensitive to metalaxyl. Growers often spray mixtures using combinations of mancozeb and cymoxanil to control late blight and to obtain better overall control of foliar diseases. Although the number of applications varies, growers in the highland region frequently apply fungicides. One grower sprayed 18 times in one season (G. L. Hartman, *unpublished data).* Our data indicated that weekly applications provided adequate control. Further reductions in applications would probably be obtained if disease forecasting programs were used. Further losses may be minimized by selecting partially resistant lines or varieties. The commercial variety used in our study (TA4) was much more susceptible with higher yield losses than L1197. Further characterization of late blight resistance in *Lycopersicon* spp., as was done initially by Richards et al. (13), is warranted as more accessions and species are now available.

LITERATURE CITED

1. Bruck, R. I., Fry, W. E., and Apple, A. E. 1980. Effect of metalaxyl, an acylalanine fungicide, on developmental stages of *Phytophthora infestans*. Phytopathology 70:597-601.
2. Caten, C. E., and Jink, J. L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Can. J. Bot. 46:329-348.
3. Dhingra, O. D., and Sinclair, J. B. 1985. Basic Plant Pathology Methods. CRC Press, Inc., Boca Raton, Fla.
4. Dowley, L. J., and O'Sullivan, E. 1981. Metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. Potato Res. 24:417-421.
5. Gallegly, M. E., and Galindo, J. 1958. Mating types and oospores of *Phytophthora infestans* in nature in Mexico. Phytopathology 48:274-277.
6. Gallegly, M. E., and Marvel, M. E. 1955. Inheritance of resistance to tomato race O of *Phytophthora infestans*. Phytopathology 45: 103-109.
7. Giddings, N. J., and Berg, A. 1919. A comparison of the late blights of tomato and potato. Phytopathology 9:209-210.
8. Hartman, G. L., Huang, Y. H, and Lee, L. H. 1991. Differentiation of *Phytophthora infestans* and *P. capsici* on detached tomato leaves. (Abstr.) Plant Prot. Bull. (Taiwan) 33: 430.
9. Ho, H. H. 1990. Taiwan *Phytophthora*. Bot. Bull. Acad. Sin. 31:89-106.
10. Hohl, H. R., and Iselin, K. 1984. Strains of *Phytophthora infestans* from Switzerland with A2 mating type behavior. Trans. Br. Mycol. Soc. 83:529-530.
11. Miller, P. M. 1955. V-8 juice agar as a general-purpose medium for fungi and bacteria. (Abstr.) Phytopathology 45:461-462.
12. Pappas, A. C. 1985. Metalaxyl resistance in *Phytophthora infestans* on greenhouse tomato in Greece. Plant Pathol. 34:293-296.
13. Richards, M. C., and Barratt, R. W. 1946. A portrait survey of the genus *Lycopersicon* for resistance to *Phytophthora infestans*. Plant Dis. Rep. 30:16-20.
14. Sawada, K. 1919. Descriptive catalogue of the Formosan fungi I. Spec. Bull. Agric. Exp. Stn. Gov. Formosa 19.
15. Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.
16. Shattock, R. C., Shaw, D. S., Fyfe, A. M., Dunn, J. R., Loney, K. H., and Shattock, J. A. 1990. Phenotypes of *Phytophthora infestans* collected in England and Wales from 1985 to 1988: Mating type, response to metalaxyl and isoenzyme analysis. Plant Pathol. 39:242-248.
17. Spielman, L. J., Drenth, A., Davidse, L. C., Sujkowski, L. J., Gu, W., Tooley, P. W., and Fry, W. E. 1991. A second world-wide migration and population displacement of *Phytopthora infestans*? Plant Pathol. 40:422-430.
18. Thurston, H. D. 1957. The culture of *Phytophthora infestans*. (Abstr.) Phytopathology 47:186.
19. Tooley, P. W. 1988. Use of uncontrolled freezing for liquid nitrogen storage of *Phytophthora* species. Plant Dis. 72:680-682.
20. 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.
21. Vartanian, V. G., and Endo, R. M. 1985. Overwintering hosts, compatibility types, and races of *Phytophthora infestans* on tomato in southern California. Plant Dis. 69:516-519.
22. Waterhouse, G. M. 1963. Key to the species of *Phytophthora* de Bary. Mycol. Pap. 92. Commonw. Mycol. Inst., Kew, England.