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

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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

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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