



Evaluation of host susceptibility, pathogen aggressiveness and sporangial survival in soil as factors affecting incidence of potato tuber infection by *Phytophthora infestans* in Ecuador

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ABSTRACT

Incidence of potato tuber infection by *Phytophthora infestans* is low in Ecuador. Factors considered to potentially affect the incidence of tuber infection include pathogen aggressiveness, host resistance, direct suppression from biological and chemical characteristics of soil acting on pathogen propagules, and exclusion resulting from soil structure and high ridging. In this study, we tested the hypothesis that low incidence could be due to reduced pathogen aggressiveness and/or low host susceptibility by comparing several pathogen isolates and commonly grown potato cultivars from Ecuador with isolates and cultivars from Europe, where tuber blight is known to be a problem. Additionally, in Ecuador, whole tubers and slices of common varieties were inoculated with local isolates of *P. infestans* to test for potential infection under Ecuadorian conditions. All isolates, regardless of origin, caused tuber infection. The aggressiveness of isolates varied, but this was both between and among Ecuadorian and Swedish isolates and it was not possible to establish a clear difference in the degree of infection based on isolate origin, or origin of potato variety. In general, we found no evidence to suggest that low aggressiveness of the pathogen or extreme resistance of the host explains low incidence of tuber blight in Ecuador. Therefore, we conclude that low incidence of tuber blight in Ecuador is probably caused by soil factors. Furthermore, exclusion due to soil structure and high hilling may play an important role as a preliminary soil infectivity study demonstrated that *P. infestans* sporangia were infective in six Ecuadorian field soils for at least 15 days.

Key words: Late blight, soil suppressiveness, tuber resistance, tuber slice assay, whole tuber assay.

INTRODUCTION

Potato (*Solanum* spp.) is an important crop in Ecuador, with about 50,000 ha planted every year, mainly on the steep slopes of the inter-Andean valleys. Yields are highly variable due to many biotic, abiotic and social constraints. According to FAO statistics the annual national average potato yield in Ecuador fluctuates around 7 t/ha (<http://faostat.fao.org/>, March, 15, 2010). Foliar late blight caused by *Phytophthora infestans* (Mont.) de Bary (1876) is generally considered the most important biotic constraint on potato yield in Ecuador (Kromann et al., 2008a). Direct losses due to this disease are difficult to estimate, but fungicide usage patterns and limited surveys indicate that foliar late blight causes significant yield losses and this form of blight is the most important disease of potato in Ecuador (Oyarzún et al., 2005).

Phytophthora infestans also infects tubers and tuber blight is common in rain-fed production systems in North America (Dorrance & Inglis, 1998) and Europe (Schepers

& van Soesbergen, 1995; Bain & Moeller, 1999). It is assumed that sporangia produced in the infected canopy are washed from the foliage and come in contact with tubers in the soil directly or as zoospores via rain water. Infection of tubers most often happens through wounds and eyes, but also through lenticels and rarely through the stolon attachment end (Hirst et al., 1965; Lapwood, 1977; Pathak & Clarke, 1987; Peters et al., 1999). A recent study done in Ecuador showed a high incidence of *P. infestans* on preemerged potato sprouts, indicating that sprouts may be more susceptible than tubers (Kromann et al., 2008b).

In spite of the importance of foliar late blight in Ecuador, there is increasing evidence that tuber blight is rare in this country (Garzón & Forbes, 1999; Oyarzún et al., 2005). Significant levels of tuber blight have not been found even though conditions would appear to favor the disease. Although fungicides are commonly used, Ecuadorian farmers generally do not fully control late blight throughout the season and some foliar infection throughout the growing period is common, indicating that inoculum

should be present during the period of tuber development. Furthermore, heavy rainfalls are common during the potato growing period, which should provide conditions favorable for spores to wash down to tubers. Ecuadorian farmers also do not generally remove or destroy foliage prior to harvest and it would appear possible that infected foliage could contaminate tubers at harvest. In addition, the fungicides that are known to be most effective in reducing tuber blight, such as fluazinam (Schepers & van Soesbergen, 1995), are not widely used in Ecuador (Ortiz & Forbes, 2003).

In general, the relationship between severity in foliage and severity in tubers is not clear; often one form of the disease occurs without the other (Schepers & van Soesbergen, 1995). Nonetheless, tuber blight is an important problem in parts of North America (Dorrance & Inglis, 1998) and Europe (Schepers & van Soesbergen, 1995; Bain & Moeller, 1999), and the very low incidence found in Ecuador is rare. For this reason, Ecuador presents an interesting case for studying factors that may limit tuber blight. Many potential factors have been proposed, including soil suppressiveness, based on soil biology, structure and/or chemistry, host resistance, lack of pathogen aggressiveness on tubers, and cultural practices within the potato production system (Oyarzún et al., 2005). A preliminary study found that the high-organic-matter andisol soils used for potato production in Ecuador were suppressive both biologically and chemically, although it was not clear which specific characteristics of the soil caused suppressiveness (Garzón & Forbes, 1999). In that study, infectivity of sporangia in six Ecuadorian soils decreased sharply after 8 days, but the rate of decrease was reduced by pasteurizing soils, indicating that some suppressiveness is potentially due to biological factors.

Of the many factors that may influence tuber blight severity, two that appear most tractable are host resistance and pathogen aggressiveness, as they can be evaluated, at least to some extent, in the absence of the biologically and physically complex soil system. The potato varieties grown in Ecuador are for the most part unique to that country. Some varieties are *native*, i.e. they are not the product of a breeding program and their time under cultivation is not known, but most native varieties belong to clonal lineages that are centuries old. The exact genetic makeup of Ecuadorian native varieties is unknown, but the situation is probably analogous to Peru where genotypic mixtures are frequently grown (Haan, 2009). However, most of the production area is now dedicated to varieties that have been bred, primarily by the national potato program, or that were introduced from the International Potato Center (CIP); this change from native to bred varieties has occurred in the last 50 years. We are unaware of previous tests to confirm that tubers of Ecuadorian varieties are susceptible to *P. infestans*. For some time, the most commonly accepted hypothesis held that *P. infestans* was introduced into the Andes after its appearance in Europe in the 1840s (Goodwin, 1996). However there is a body of literature suggesting a South

American origin for the pathogen (see Gomez-Alpizar et al., 2007), which could indicate that resistance to tuber infection by *P. infestans* could have evolved in South American germplasm, particularly in native varieties that may have coevolved with the pathogen.

There is also reason to suspect a reduced capacity in the pathogen for tuber infection. The pathogen populations in Ecuador and other highland tropical areas are subject to unique selection pressure as the foliar phase of the disease is present year-round and primary inoculum appears to come from other fields via air and not via infected tubers (Garrett et al., 2003), which is an important mechanism of initial infection in temperate zones (Zwankhuizen et al., 1998). Capacity to infect tubers would seem to provide little advantage to a particular genotype within the Ecuadorian pathogen population.

In this paper we examine host susceptibility and pathogen aggressiveness on tubers and assess their possible role in tuber blight development in Ecuador. Specifically, we tested the null hypotheses that i) isolates from Ecuador and Europe are similar in their ability to cause tuber blight and ii) potato genotypes from Ecuador and Europe have similar susceptibility to tuber infection by *P. infestans*. Rejection of either one of these hypotheses could be considered a strong indication of a potential cause of low incidence of tuber blight in Ecuador. We also present data from a study on tuber blight incidence in an experimental field and data from a bioassay on sporangial survival in several Ecuadorian soils, for which a preliminary report was published previously (Garzón & Forbes, 1999).

MATERIALS AND METHODS

Incidence of tuber blight

Incidence of tuber blight was measured in the experimental station of the International Potato Center south of Quito, Ecuador (CIP-Quito), where three potato genotypes (Papa Pan, C-144 and LBR-37) were grown in a trial designed to evaluate the effect of host diversity on disease severity, the results of which were published previously (Pilet et al., 2006). All three potato varieties are introduced and come from CIP's potato breeding program. They would be categorized as bred potatoes in the classification we present in the introduction. Plant spacing was 80 cm between rows and 30 cm between plants, which represents a planting density that is recommended for potato seed cultivation and which is higher than the density used in traditional potato production in Ecuador. Mixed variety plots were used in the earlier study on host diversity (Pilet et al., 2006), but for the current assessment of tuber blight incidence we only sampled single-genotype plots (i.e., single genotype plots of Papa Pan, C-144 or LBR-37). In the field trial all plots were farmed following traditional Ecuadorian potato production practices, which include high ridging done manually, leaving a minimum distance from the bottom to the top of the ridges of minimum 0.5 m. In

total, over 9000 tubers were sampled at harvest, with about 3000 coming from each host genotype (Table 1). Samples were taken at harvest and represented a stratified-random selection of tubers in that relatively even numbers were taken from replicates in the trial. Three fungicide regimes (no spray, weekly and every two weeks) were used in the host diversity study and tubers were sampled from the three treatments, although the exact proportions of each treatment are not known. Nonetheless, sampling from all three fungicide treatments insured variable levels of foliage late blight severity. Tubers were harvested, washed and evaluated for visual symptoms of tuber blight. If a tuber had symptoms that could potentially be caused by *P. infestans*, a slice (approx. 1 cm) from the symptomatic area was incubated for one week in a high-humidity chamber at 15°C. Infection was confirmed by detection of *P. infestans* mycelia or sporangia on the incubated tuber slices.

Tuber susceptibility of Ecuadorian varieties inoculated with Ecuadorian *P. infestans* isolates

The susceptibility to *P. infestans* of four of Ecuador's most widely-grown varieties was assessed both on tuber slices and on whole tubers. Three of the four varieties, Gabriela, Superchola and Esperanza, were chosen because they are widely grown and highly susceptible to foliage infection by *P. infestans*. The fourth, Frippapa, has a moderate level of resistance to foliage infection. There are no widely-grown varieties with a high level of foliage resistance.

Inoculum. Sporangia used for the assessment came from leaflets with newly-sporulating lesions that were collected from a potato crop at the CIP-Quito station several days before the experiment. Leaflets were gently rinsed, disinfested in 70% ethanol for a minute, rinsed twice in sterile water and dried with paper towels. The infected leaf tissue was then incubated in closed humid chambers (small plastic boxes lined with moist paper) at 15°C in the dark for 3 to 4 days. Sporangia were collected in sterile water and suspensions adjusted to 1×10^4 sporangia/mL using a hemacytometer. Inoculum was assumed to be composed entirely of the EC-1 clonal lineage of *P. infestans*, which is the dominant lineage on potato in Ecuador (Forbes et al., 1997; Oliva et al., 2007).

Tuber slice assay. Four uniform tubers of each of the potato varieties, Frippapa, Gabriela, Superchola and Esperanza were superficially disinfested by dipping in 70% alcohol for 1 min, then briefly in 90% alcohol and dried in a laminar flow hood. Three 7-mm slices per tuber were cut with a sterilized knife (12 slices per variety). Each 12-slice cohort constituted an experimental unit and was placed on a metal wire mesh in a plastic container (30 x 15 cm) lined with moist paper. One side of the tuber slices was then sprayed with the inoculum using an atomizer. The volume per area was not measured but enough *P. infestans* suspension was

used to lightly cover each slice. The slices were incubated for seven days at 15°C in the dark. Each experimental unit was replicated three times. The percentage area of tuber slice colonized by *P. infestans* was estimated in each container using the procedure described by Dorrance and Inglis (1998). Varieties were compared using a least significant difference test following a one-way analysis of variance.

Whole tuber assay. The same four varieties described above were tested for susceptibility using whole tubers. For this assay, 90 tubers of each variety were surface disinfested as described above. The tubers from each variety were then randomly divided into groups of 30 tubers, each of which served as one experimental unit. Each experimental unit was sprayed with approximately 30 mL of inoculum; during spraying, tubers were rotated to increase the area covered. Tubers were incubated in darkness in plastic boxes lined with moistened towel paper at 15°C for seven days. After incubation tubers were evaluated visually and also cut into slices to improve identification of symptoms. The number of tubers with symptoms of tuber blight per experimental unit was registered. Variety means were compared with Duncan's multiple range test following one-way analysis of variance.

Comparison of aggressiveness of Ecuadorian and Swedish *P. infestans* isolates

To explicitly test the null hypothesis that isolates from Ecuador and Europe are similar in their aggressiveness on potato tubers, we compared two Ecuadorian isolates with four isolates from Sweden.

P. infestans isolates. The aggressiveness of six *P. infestans* isolates was evaluated at the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. Four came from farmers' fields in southwest Sweden in 2001, of which two were of the A1 mating type and two of the A2 mating type. The two from Ecuador were both A1 mating type and belonged to the EC-1 clonal lineage. Both were collected at the CIP-Quito experiment station, one in 1997 from an experimental breeding line (Isolate 3063) and the other in 2001 from the CIP variety LBR-37 (Isolate LBR-37). All isolates were compared both on tuber slices and whole tubers of the variety King Edward.

Inoculum. To insure isolates had not lost pathogenicity, inoculum was multiplied on tuber slices of variety Bintje for five cycles after cultures had been taken from storage on agar, and then adjusted to a concentration of 1×10^4 sporangia/mL. Separate inoculum batches of each isolate were used for the whole-tuber and tuber-slice assays.

Whole-tuber assay. The variety King Edward was used and all tubers were cleaned with water, dipped in 90% alcohol and dried in a laminar flow hood before use. The sporangial

suspension was sprayed on the tubers, which were turned while spraying to ensure coverage. For each isolate in the tuber assay, 12 whole tubers were placed in a plastic box lined with moist paper representing an experimental unit. Each experimental unit (isolate-variety combination) was replicated three times. After inoculation, tubers were incubated for three weeks at 15°C in a moist chamber. Tubers were evaluated visually and also cut into slices to improve identification of symptoms. The number of tubers with symptoms of tuber blight per isolate was registered and means were compared with Duncan's multiple range test following one-way analysis of variance.

Tuber slice assay. Prior to use, all tubers of King Edward were cleaned with water, dipped in 90% alcohol, dried in a laminar flow hood and sliced as described in the tuber slice assay above. A sporangial suspension of each isolate was sprayed on one side of 50 slices. Tuber slices were then incubated for seven days at 15°C in plastic boxes lined with moist paper. Colonization of each slice was visually estimated on one assessment day (seven days after inoculation) using a semi-quantitative scale of severity classes ranging from 0 to 5; where 0 represented no visible mycelial growth, 1 represented 0 - 25%, 2 represented 25 - 50%, 3 represented 50 - 75%, 4 represented 75 - 100% and 5 indicated that 100% of the slice was overgrown with *P. infestans* mycelia.

Comparison of Ecuadorian and European potato varieties for susceptibility to *P. infestans* in tubers

To explicitly test the null hypothesis that cultivars from Ecuador and Europe are of similar susceptibility to tuber infection by *P. infestans*, cultivars from Sweden and Ecuador were compared simultaneously in Sweden. Two Ecuadorian varieties, Superchola and Esperanza, and two European varieties, Bintje and Asterix, were compared for susceptibility to *P. infestans* in tubers. All plants were produced in a greenhouse in Sweden and harvested after approximately four months. Production was done in the winter season, and only a few small tubers of the Ecuadorian short-day varieties were available. It was therefore decided only to carry out a tuber slice assay. Twelve tuber slices of each variety were inoculated with isolate 3063 from Ecuador and 12 additional slices with isolate 39 from Sweden as described in the tuber slice assay above. Inoculum production and incubation were done as described above for the tuber slice assay comparing Ecuadorian and Swedish isolates of *P. infestans*. Due to the small diameter of tuber slices, growth of *P. infestans* on each tuber was not quantified, but rather the incidence of visible signs or symptoms was recorded.

Infectivity of *P. infestans* sporangia in Ecuadorian soils

Six fields differing in their content of organic matter, aluminum and pH were sampled from five Ecuadorian provinces with important potato production (Table 4). Soil

samples were sieved to an average aggregate size of 2 mm and then infested with a sporangia suspension of *P. infestans* at a concentration of 2×10^4 sporangia per cubic centimeter soil, while soil water content was adjusted to field capacity and maintained at that level. The sporangial suspension was prepared using sporangia from a cultured isolate (no. 2667; EC-1), multiplied on leaflets of susceptible potato variety Chata Blanca for three cycles to produce enough inoculum for the experiment. Control soils were initially mixed with sterile water and thereafter treated the same as infested soils. Control and infested soils were kept in closed containers and maintained at an ambient temperature ranging from 10 to 25°C for the duration of the assay. The capacity of the inoculum in the soil to infect tuber slices of the susceptible variety Uvilla was evaluated at 8, 15 and 30 days after soil infestation. At each time, superficially sterilized tubers were cut in 5 mm slices and a sample of 0.3 mL of the infested soil was spread to completely cover the slice. A second slice was put on top of the soil layer. Slices were incubated in humid chambers at 15°C in the dark. Five slice-soil "sandwiches" represented an experimental unit (one per soil by incubation-time combination) and each unit was replicated three times; randomly selected sub-samples of soil were used for each replicate. Infectivity was measured after a seven-day incubation period as the percentage of "sandwiches" with visible *P. infestans* mycelium. For each number of days after soil infestation, infection percentages were compared with Duncan's multiple range test following one-way analysis of variance. Control soils were not included in the analysis of variance as they did not represent a viable infestation.

RESULTS

Incidence of tuber blight in the field

Severity of foliage blight was variable in the plots from where tubers were sampled, but in general disease pressure was high as unsprayed plots of the susceptible variety LBR-37 reached 100% within 100 days from planting. Disease in the unsprayed plots of the resistant varieties was between 5 and 20% (Pilet et al., 2006). Nonetheless, incidence of tuber blight was low (near 1%) in all three varieties, and was even somewhat higher (1.2%) in the foliar resistant variety Papa Pan (Table 1).

Tuber susceptibility of Ecuadorian varieties inoculated with an Ecuadorian *P. infestans* isolate

Whole tubers and slices of four widely grown Ecuadorian varieties became infected after inoculation with a suspension of sporangia. After seven days of incubation at 15°C, all tuber slices showed signs of the pathogen and were covered on the average between 40 and 77% by *P. infestans* mycelial growth, depending on variety (Table 2). There was a tendency in both whole tubers and slices for somewhat more infection in variety Frippa and reduced infection in variety Superchola, but differences were

TABLE 1 - Incidence of tubers infected with *Phytophthora infestans* sampled from an experimental field, Quito, Ecuador

Variety	No. tubers sampled	Tubers with potential blight symptoms	Tubers with blight infections confirmed	Infected tubers (%) ¹
Papa Pan	2855	94	36	1.2
C-114	2753	95	16	0.6
LBR-37	2934	73	6	0.2
Total / average	8542	262	58	0.7 ²

¹Percentage of infected tubers relative to the complete sample

²Average percentage infected tubers in three varieties.

TABLE 2 - Percentage of tuber slices covered with *Phytophthora infestans* mycelial growth and number of tubers with blight symptoms in two separate assays designed to test susceptibility to *P. infestans* of tubers of popular Ecuadorian potato varieties

Variety	Coverage of tuber slices (%)		Number of tubers with blight symptoms	
	Average ¹	Standard deviation	Average ²	Standard deviation
Fripapa	77 ^a	23.1	3.0 ^a	1.0
Gabriela	63 ^{ab}	23.1	2.3 ^a	0.6
Superchola	40 ^b	10.0	0.7 ^a	1.2
Esperanza	40 ^b	10.0	2.7 ^a	4.6

¹Average of 12 slices per each of three replicates; numbers followed by different letters are statistically different at $P = 0.05$ based on a least significant difference test.

²Average number of tubers with symptoms per 30-tuber replicate; numbers followed by different letters are statistically different at $P = 0.05$ based on a least significant difference test.

not statistically significant due to high variation among replicates (Table 2). Nevertheless, it is worth noting that Superchola is more susceptible than Fripapa in foliage. The frequency of symptoms was much lower in whole tubers, where the number of infected tubers per 30-tuber-replicate did not go above three, although all varieties had some infection.

Comparison of aggressiveness of Ecuadorian and Swedish *P. infestans* isolates

Both Swedish and Ecuadorian isolates caused infection on tuber slices and whole tubers (Table 3). Isolates varied for their aggressiveness but there was no clear pattern related to country of origin. The two isolates from Ecuador varied greatly for the proportion of inoculated tuber slices that fell into different severity classes; however, the same was true for the Swedish isolates; two of them had no slices in the highest categories. For the whole tuber assay, the Ecuadorian isolates were among the lowest in incidence of tubers infected, but did nonetheless cause disease (Table 3). Based on these results, we cannot reject the hypothesis that isolates from Ecuador and Europe (Sweden) are similar in their capacity to infect tubers.

Comparison of Ecuadorian and European potato varieties for susceptibility to *P. infestans* in tubers

All slices of each variety showed signs and/or symptoms of tuber blight, regardless of the isolate used.

Although intensity of infection was not quantified, there was no clear difference among the varieties under the conditions of this experiment, so we could not reject the null hypothesis that cultivars from Ecuador and Sweden are of similar susceptibility to tuber infection by *P. infestans*.

Infectivity of *P. infestans* sporangia in Ecuadorian soils

All infested soils remained infective for up to 15 days and some infectivity could be measured in three soils after 30 days (Table 4). Control soils were not infective. There was no clear relationship between soil characteristics and infectivity. For example, there were two soils with high pH and low organic matter, which would not be considered typical andisols from potato growing regions. One of these had the highest infectivity (Pichincha) and the other the lowest (Tungurahua2) (Table 4).

DISCUSSION

Our evaluation of incidence of tuber infection in an experimental field in CIP-Quito was limited in scope but nonetheless gave results consistent with a much larger study done in Ecuador several years ago (Oyarzún et al., 2005). In both studies the average incidence was less than 1%, but in the current study on variety Papa Pan there was an incidence slightly over 1%. Thus, in both studies, tuber blight was found, but at low levels. In both studies foliage blight was severe. In the present study, only tubers with

TABLE 3 - Comparison of aggressiveness of six isolates of *Phytophthora infestans*, four of which were from Sweden and two from Ecuador, inoculated on tuber slices and whole tubers of potato variety King Edward

Isolate	Mating Type	Origin	Number of tuber slices in severity classes ¹					Incidence, whole tubers ²	
			Class 0	Class 1	Class 2	Class 3	Class 4		Class 5
LBr-37	A1 ³	Ecuador	1	1	10	8	10	20	3.1 ^b
3063	A1	Ecuador	6	33	11	0	0	0	4.2 ^{ab}
55	A2	Sweden	0	2	5	8	12	23	6 ^a
12	A1	Sweden	8	29	9	2	2	0	6 ^a
39	A1	Sweden	1	32	11	1	0	0	4.4 ^{ab}
35	A2	Sweden	2	38	5	0	0	0	3.1 ^b

¹Colonization of each slice was visually estimated using a semi-quantitative scale from 0 to 5, where 0 represented no growth and 5 indicated that the slice was overgrown with *P. infestans* mycelium.

²Number of tubers showing symptoms of 12 inoculated per three replicates; numbers followed by different letters are statistically different at $P = 0.05$ based on a least significant difference test.

³The Ecuadorian isolates belong to the dominant clonal lineage on potato in Ecuador, EC-1.

TABLE 4 - Survival of sporangia of *Phytophthora infestans* in six field soils from Ecuador

Origin	Altitude (m.a.s.l.)	Soil type	Organic matter (%)	pH	Aluminum (Meq/100g)	Infection (%) at		
						8d	15d	30d ¹
Carchi	3400	Loam Sand	17.9	4.4	2.9	100	67 ^{ab}	0 ^b
Chimborazo	2740	Loam Silt	12.4	6.0	0.4	100	100 ^a	7 ^b
Pichincha	2500	Loam Sand	2.2	6.8	0.4	100	100 ^a	33 ^a
Loja	2750	Loam Clay	7.8	4.7	2.3	100	93 ^a	7 ^b
Tungurahua1	3310	Loam Silt	15.4	5.4	0.8	100	40 ^{bc}	0 ^b
Tungurahua2	2900	Loam	3.7	7.5	0.3	100	13 ^c	0 ^b

¹Numbers followed by different letters are statistically different at $P = 0.05$ based on a least significant difference test.

some symptoms were incubated, so infections occurring at or just before harvest may have been missed, although we assume that if we had identified the latest infections this would not have significantly changed results. Our study was also consistent with the idea that many aspects of foliage and tuber blight appear to act independently. We had very little foliage blight in Papa Pan (see Pilet et al, 2006) but had the highest level of tuber blight in this cultivar. It is not known if this independence of infection severity in tubers and foliage is due to genetic or other reasons.

In this paper we evaluated susceptibility of Ecuadorian potato varieties and compared Swedish isolates of *P. infestans* (as representatives from a European country where tuber blight occurs regularly) with Ecuadorian isolates in an effort to determine whether host or pathogen might explain low levels of tuber infection found in Ecuador. We found that all the Ecuadorian varieties tested with an Ecuadorian isolate were readily infected in a standard tuber slice technique and were also infected, although to a lesser degree, when whole tubers were inoculated. The degree of infection in tuber slices was similar to that reported by

Dorrance and Inglis (1998), indicating that the varieties are susceptible to tuber infection under the conditions of the test that was used. The whole tuber evaluation done here cannot be readily compared with that of the earlier study because different response variables were measured. The isolates from the Ecuadorian pathogen population also performed in a similar way to Swedish isolates when tested together in Sweden, and there was more variation within country populations than between them. Based on these results, we cannot reject the hypothesis that pathogen isolates are similar for infectivity in Ecuador and Sweden, nor can we reject the hypothesis that cultivars are similar between Ecuador and Europe for infectivity. Therefore, we could not find clear evidence in the host or pathogen that explains low tuber blight incidence in the field in Ecuador.

Nonetheless, the tests we conducted were done in the laboratory, which may not fully reflect field conditions. For example, variety resistance in the field may also be a result of characteristics other than direct resistance to infection. Long stolons, characteristic of some of the native Andean varieties, could place tubers farther from the stem

and reduce the chances of spores coming into contact with tubers.

Likewise, the standard tuber slice techniques used in our studies did not include initial infection through the tuber skin and, therefore, did not fully reflect the conditions of tuber infection on whole tubers. Dorrance & Inglis (1998) compared two laboratory methods for assessing tuber blight resistance and found that the whole tuber as well as the tuber slice method successfully separated resistant from susceptible cultivars, although the tuber slice method had more variation within and among experiments. In order to reduce variation we used calibrated sporangial solutions instead of agar plugs to inoculate tuber slices. Although the modified tuber slice method may be used to assess open-wound tuber susceptibility and aggressiveness of *P. infestans* isolates in tuber flesh tissue, if sufficient tubers are available, more reliable whole tuber assays should be used to reflect field conditions (Dorrance & Inglis, 1998), since tuber skin and cortex may have an important role in tuber-blight resistance.

Soil suppressiveness to *Phytophthora* has been associated with both biotic (Broadbent & Baker, 1974; Lozoya-Saldaña et al., 2006; McDonald et al., 2007) and abiotic soil factors (Ko & Shiroma 1989; Andrivon, 1995a). Suppression of *P. cinnamomi* has been associated with high content of organic matter and exchangeable Ca and N levels, which principally suggests high antagonistic biological activity in suppressive soils (Broadbent & Baker, 1974). Typically, the main highland potato-growing regions of Ecuador have black Andean soils, known as andisols (USDA soil taxonomy) or andosols (FAO soil classification) (Buytaert et al., 2006). They are derived from volcanic ash and contain high levels of organic matter, low pH and high levels of exchangeable Fe, Ca and Al (Wada, 1985). Suppression of *P. infestans* has been associated with high organic matter (Andrivon, 1994a) and low pH (Andrivon, 1994a; Andrivon, 1994b); factors which may increase levels of antagonistic bacteria (Lozoya-Saldaña et al., 2006). However, low pH might also decrease the infectivity of *P. infestans* inoculum, and aluminum sulfate or aluminum chloride can inhibit mycelial growth of *P. infestans* directly (Andrivon, 1995b). It has been estimated that inorganic soil particles supported by low pH are responsible for *Phytophthora* suppression in volcanic soils in Hawaii (Ko & Nishijima, 1985).

Nonetheless, although Ecuadorian andisols are likely to contain biotic and abiotic *P. infestans* suppressing factors, we found that sporangia remained viable in the six Ecuadorian soils for at least 15 days when using a concentration of 2×10^4 sporangia per cubic centimeter soil and differences among soils could not clearly be associated with organic matter, pH or aluminum. Moreover, it is worth noting that incidence of the soil borne pathogen *Rhizoctonia solani* is high in Ecuadorian potato fields (Fankhauser, 2000).

Natural levels of sporangia in soil are not known; therefore, it is possible that our test underestimated the

suppressive qualities of the soils by using excessive amounts of inoculum. However, a recent study in Ecuador in which natural spore deposition was shown to cause infection in pre-emerged sprouts is consistent with the hypothesis of sporangial survival in these soils (Kromann et al., 2008b). Furthermore, our study demonstrated high levels of infectivity after 8 days, which may indicate that some infection would have occurred even with lower inoculum densities.

Overall, our studies indicate that tuber infection in Ecuador does not appear to be highly constrained by host resistance and pathogen aggressiveness. Additionally, our preliminary soil infectivity study suggests that a high level of soil suppressiveness does not occur in Ecuadorian soils; although this factor should be studied in much greater detail. This then leads to the question of what does cause the relatively low levels of tuber infection we found herein and that was reported by Oyarzun et al (Oyarzún et al., 2005). As noted earlier, high ridging done traditionally by Andean farmers has been cited as a tuber blight suppressing factor (Porter et al., 2005). This certainly appears logical and is even more apparently plausible when one considers that ridging is done with andisols that have small soil pore size and do not crack even upon drying (Knapp, 1991).

It is also highly probable that low frequency of tuber blight in Ecuador results from the additive effects or synergistic interaction of multiple factors, including interactions between soil microorganisms and inhibitory compounds in the soil, soil structure, high ridging, climate (high solar irradiance), harvesting methods and others. In a multiple-factor scenario, one can easily imagine that a factor of small effect that we could not measure (e.g., a pathogen population slightly less adapted to tuber infection) could also play a role.

Finally, although there is evidence that tuber blight incidence is low in Ecuador, it is probably not as low as indicated by our data. Recent studies in Ecuador (Kromann et al., 2008b) and elsewhere (Hussain et al., 2005; Johnson & Cummings, 2009) have indicated that tubers can be latently infected and not identified in classical tuber blight surveys. Symptomless tubers caused about 1% infection in a recent study when planted in a greenhouse in Ecuador (Kromann et al., 2008b), although the sample size was very small. This would seem to indicate that tuber infection in Ecuador, including both symptomatic and latent, is higher than previously thought, albeit probably lower than in other parts of the world.

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