Short Communication

US-8 and US-11 Genotypes of *Phytophthora infestans* from Potato and Tomato Respond Differently to Commercial Fungicides

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ABSTRACT

Isolates of *Phytophthora infestans* collected in Canada in 1997 from both potatoes and tomatoes, were tested on potato leaf discs for their response to an equal active ingredient concentration (10 μg a.i./mL) of the following commercial fungicides: Acrobat MZ (Dimethomorph and Mancozeb), Ridomil Gold (metalaxyl-m), Dithane (Mancozeb), Curzate (Cymoxanil), Bravo (Chlorothalonil), and Tattoo C (Propamocarb and Chlorothalonil). Relative percent leaf infection values, estimated on fungicide-treated vs fungicide-free leaf discs, were compared among isolates from the US-8 and US-11 genotypes isolated from the two host plants. Based on an equal concentration of each fungicide's active ingredients, variations in relative percent leaf infection were recorded between US-8 and US-11 genotypes, and between potato and tomato isolates within each genotype. Bravo and Tattoo C used with similar active ingredients concentrations were the most inhibitory to all groups of isolates. Dithane and Ridomil Gold provided uniform low inhibition against *P. infestans* when tested on potato leaf discs. The different behavior of *P. infestans* isolates from potato vs tomato suggests that management of late blight in these two important crops must take such differences into consideration. In particular, the nature and concentration of the fungicides to be applied must take into account any information available about genotypes present on each crop.

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RESUMEN

Aislamientos de *Phytophthora infestans* colectados de papa y tomate en Canadá durante 1994 a 1998 fueron probados para su respuesta *in vitro* a iguales concentraciones de ingrediente activo (1,2.5y 5ug i.a./mL) de seis fungicidas comerciales: Acrobat MZ (dimethomorph y mancozeb), Ridomil Gold (metalaxyl-m), Dithane (mancozeb), Curzate (cymoxanil), Bravo (chlorothalonil), y Tattoo C (propamocarb y chlorothalonil). Se estimó el crecimiento miceliano relativo en medio de cultivo con y sin fungicida entre los aislamientos de los gentipos US-8 y US-11 de las dos plantas hospedantes. También se estimó el efecto de estos fungicidas sobre la germinación de las esporas de los aislamientos de los dos genotipos. El crecimiento miceliano de los aislamientos US-8 y US-11 se vio afectado por Bravo y Tattoo C, seguido por Acrobat y luego por Cruzate y Dithane. El Ridomil Gold no fue efectivo en inhibir el crecimiento *in vitro* de los aislamientos US-11 a la concentración probada. Entre los aislamientos del mismo genotipo colectados en años diferentes se observaron variaciones en sensibilidad a los diferentes fungicidas. Por ejemplo, sobre la base de 2.5 y 5μg i.a./mL, los aislamientos de papa US-11 colectados de 1995 a 1997 mostraron una creciente sensibilidad a Curzate y Ridomil Gold mientras que se registró una sensibilidad decreciente al Dithane. Para los aislamientos US-8 las respuestas a los fungicidas variaron de acuerdo al fungicida y al año. Las variaciones fueron generalmente no significativas para Acrobat, Curzate, Bravo y Tattoo C en el tiempo. Contrariamente, los aislamientos de papa de los años 1995 a 1998 fueron menos sensibles al Dithane y al Ridomil Gold que los de 1994, 1996 y 1997.

INTRODUCTION

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is one of the most destructive diseases of potato crops. This disease can also affect tomato, pepper, eggplant, and various solanaceous weeds (Platt 1994). The success of integrated management of this disease depends significantly on appropriate fungicide schedules and mode of application.

When the world populations of *P. infestans* were dominated by a single genotype called US-1 (Goodwin et al. 1994b), metalaxyl-based fungicides were particularly successful in controlling this disease. By the early 1980s, new genotypes spread worldwide (Goodwin et al. 1996), and showed increasing levels of resistance to metalaxyl (Daayf et al. 2000). In North America, new genotypes, including US-6, US-7, and US-8 were detected in the early 1990s (Fry et al. 1993). In the recent years in Canada, *P. infestans* populations have been dominated by the US-8 genotype, except in British Columbia where both US-8 and US-11 have been found (Daayf et al. 2000; Platt et al. 2002). These genotypes have consistently exhibited differential responses to metalaxyl, concomitant with increasing diversity and disease incidence and severity in most potato-growing areas of the United States and Canada (Goodwin et al. 1994a).

As the capacity of metalaxyl to control late blight decreased, the chemical industries developed new fungicides in order to control this disease. However, when metalaxyl-resistant strains caused severe epidemics in the United States and Canada in 1994, no fungicide registered for late blight had post-infection efficacy (Mayton et al. 2001). In 1995, three fungicides, supposedly post-infection efficient, were registered in the United States (Mayton et al. 2001). These fungicides were Acrobat MZ (Cyanamid), Curzate M8 (Dupont), and Tattoo C (Agrevo). While many studies have examined metalaxyl resistance, which rose quickly after its commercialization, very few have determined the response of *P. infestans* to other fungicides used to control late blight in Canada and the USA (Hamm and Clough 1999; Kato et al. 1997; Mayton et al. 2001; Powelson and Inglis 1999). In addition, because more than one genotype of *P. infestans* are now involved, studies on their differential reaction to fungicides will increase our understanding of how the pathogen is able to adapt to disease management programs, and how chemical treatment practices may influence its behavior and evolution. In a former study we evaluated *in vitro* response of *P. infestans* genotypes US-8 and US-11, currently predominant in Canada, to common and recently registered fungicides: Ridomil Gold, Dithane, Curzate, Acrobat, Bravo, and Tattoo C (Daayf and Platt 2003). Interesting observations were made in the *in vitro* tests, but such results do not necessarily apply when the fungicides are used on the plant tissues. The purpose of the present study was (i) to determine and compare the response of US-11 and US-8 isolates, collected from potato and tomato, to the tested fungicides directly on potato leaf discs, and (ii) to compare these results with our recent *in vitro* assessments (Daayf and Platt 2003).

MATERIALS AND METHODS

Phytophthora infestans *Isolates*

Thirty-seven isolates of *Phytophthora infestans* were used in this study. They were obtained in 1997 from naturally infected potato leaves, stems and tubers, and from tomato leaves, stems, and fruit samples, as previously described (Daayf et al. 2000).

Response of P. infestans *Isolates to Fungicides*

The fungicides evaluated were Acrobat MZ (9% dimethomorph, 60% mancozeb), Curzate 60 (60% cyamoxanil), Ridomil Gold (49% metalaxyl-m), Dithane DF (75% Mancozeb),Bravo 500 (54% Chlorothalonil), and Tattoo C (31% propamocarb hydrochloride, 31% chlorothalonil). The response of *P. infestans* isolates to each of the several fungicides was assessed directly on leaf discs of the potato cultivar Russet Burbank grown in a greenhouse. For this purpose, leaf discs were obtained randomly from 6-7 wk growing plants. They were cut using a cork borer (#10 = 16 mm in diameter), surface-cleaned by immersion in sterile water, dried on sterile filter paper, and then immersed in the fungicide solution (10 μg a.i./mL) before being placed on wet filter paper in a sterile 9-cm Petri dish (five discs per plate). Sterile distilled water was used as the control treatment. Inoculum was prepared by washing sporangia from culture plates with sterile distilled water and adjusting suspensions to $2x10^4$ sporangia/mL using a hemacytometer. Spore suspensions were left 2.5 h at 4 C to allow release of zoospores before inoculation. Then inoculations were performed under aseptic conditions in an air-flow hood by placing a 10-μL drop of the inoculum solution on the center of each disc. Plates with inoculated discs were randomly placed in a growth cabinet (95% humidity and 15 C). Each isolate was

inoculated onto five leaf discs placed in each of three separate plates, and the experiment was repeated three times.

Response of *P. infestans* isolates to fungicides was measured using the percent leaf infection on leaf discs either treated or not with the tested fungicides. Late blight lesions were scored eight days after inoculation according to the percentage of leaf disc showing visible infection by *P. infestans*. A relative percent leaf infection (%) was obtained by dividing the percent area infected in fungicide-treated leaf discs by the percent area infected in control leaf discs. This was used to compare the response to fungicides among all isolates, or groups of isolates.

Data Analysis

Data analysis involved four pathogen isolate groups (US-11 isolates from potato [five isolates from British Columbia and Manitoba] and tomato [five from BC]; US-8 isolates from potato [24 from BC, MB, New Brunswick, Prince Edward Island, and Quebec] and tomato [five from BC and PEI]) × six fungicides and one control treatment × five leaf discs × three replications × three experiment repetitions. Data from the three experiments were combined, due to the low variability among the three experiments for each group of isolates, and all data were analyzed using analysis of variance. Means were compared based on the test of the least significant difference (LSD, P=0.05).

RESULTS AND DISCUSSION

In this study we compared six chemical fungicides based on equal concentration of active ingredients for their relative inhibition effects against *P. infestans*. Fungicides and isolates of *P. infestans* were applied directly onto potato leaf discs, in order (i) to simulate plant conditions and (ii) to compare the present data with those obtained with *P. infestans* grown on media amended with the same fungicides (Daayf and Platt 2003).

The tested fungicides had different effects on the aggressiveness of *P. infestans* genotypes.

US-8 Isolates from Potato (24 Isolates)—All tested fungicides, except Ridomil Gold, reduced the spread of *P. infestans* on leaf discs as compared to control leaf discs. Treatment of leaf discs with Tattoo C and Bravo resulted in the best protection levels with 40% and 54% late blight infection, respectively (Figure 1A). Acrobat, Curzate, and Dithane produced uniform results in terms of their effects on late blight disease expression (81%-88% infection relative to the untreated control). On the other hand, when leaf discs were treated with Ridomil Gold, infected area was greater than on the control leaf discs (Figure 1A).

US-8 Isolates from Tomato (Five Isolates)—Of the six fungicides tested, only four reduced late blight incidence on leaf discs, with Tattoo C providing the best level of late blight reduction (32% relative percent leaf infection). Tattoo C was followed by Curzate, Bravo, and Acrobat, with 51%, 61%, and 71% late blight relative infection, respectively. Leaf discs treated with Dithane or Ridomil Gold had levels of infection similar to the non-protected control (Figure 1B).

US-11 Isolates from Potato (Five Isolates)—Only Bravo and Tattoo C significantly reduced late blight infection caused by US-11 potato isolates, with 42% and 57% late blight relative percent leaf infection, respectively. The late blight reduction provided by Acrobat, Curzate, and Dithane was not significantly different from the control water treatment (Figure 1C). As with potato US-8 isolates, at the tested concentration, Ridomil Gold significantly promoted infection by US-11 potato isolates (Figure 1C). In addition, even if the results obtained with Curzate and Dithane were not statistically different from the control, the tendency was towards promoting infection (Figure 1C).

US-11 Isolates from Tomato (Five Isolates)—These isolates were relatively sensitive to all fungicides at the tested concentration. Curzate, Bravo, and Tattoo C considerably reduced infection by this group of isolates (Figure 1D). Relative percent infection values with Acrobat, Dithane, and Ridomil Gold were not significantly different from each other (Figure 1D).

As such, variations were recorded between US-8 and US-11 genotypes, and between potato and tomato isolates within a single genotype in terms of responses to the tested fungicides. Similar variations were observed with the *in vitro* tests (Daayf and Platt 2003), which confirms the validity of using the *in vitro* test. For example, Bravo and Tattoo C were consistently the most inhibitory on all groups of isolates both *in vitro* (Daayf and Platt 2003) and on the potato leaf discs. In addition, Dithane and Ridomil Gold, which had the lowest inhibitory effects when tested *in vitro*, provided a similar low level of inhibition against *P. infestans* when tested on potato leaf discs.

The capacity of *P. infestans* to cause late blight on both potato and tomato provides an interesting background to

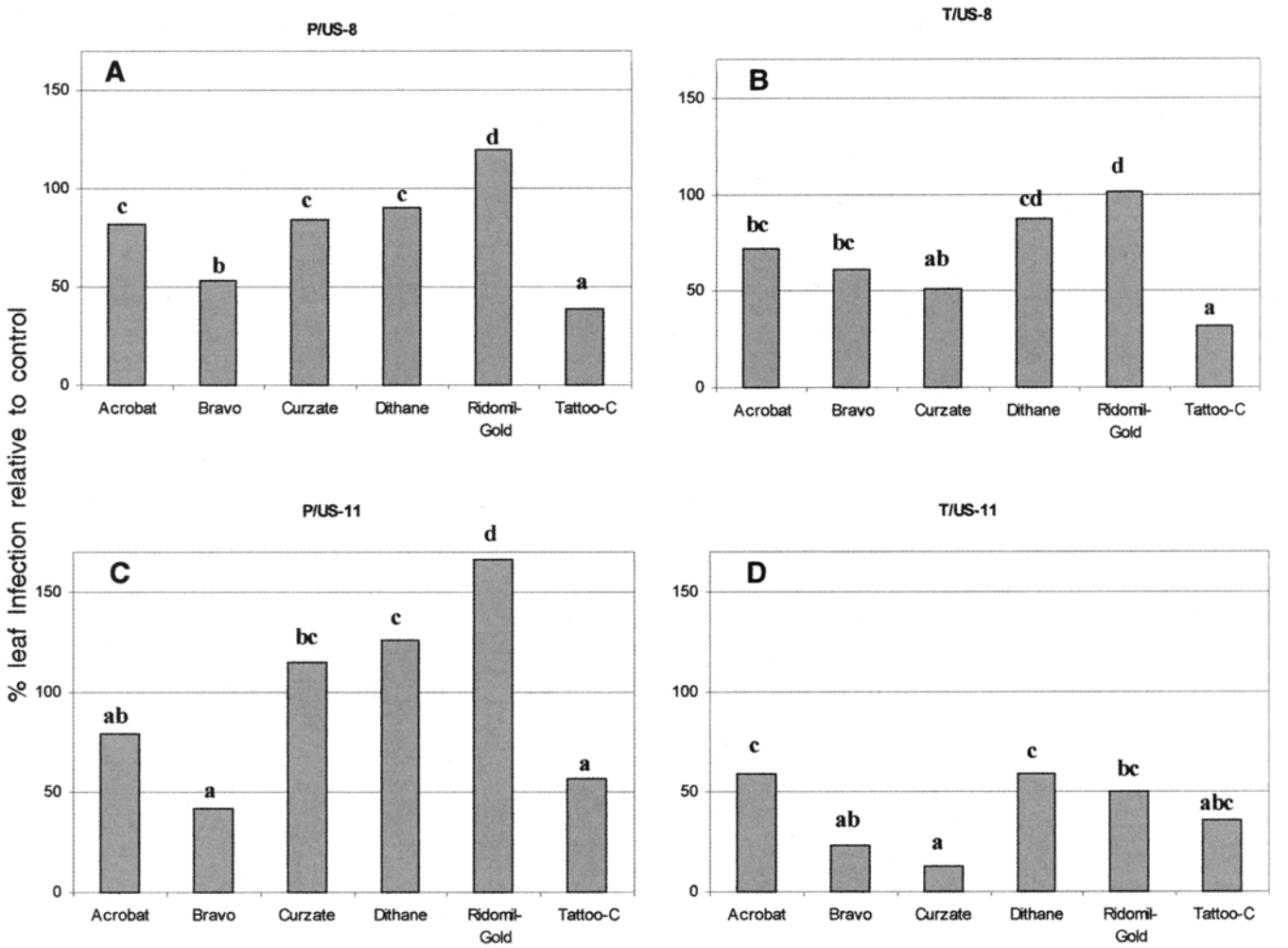

FIGURE 1.
Effect of fungicides on late blight development in potato leaf discs caused by inoculation with DUS-8C and US-11 genotypes of *P. infestans* (P = potato, T = tomato)

study late blight epidemiology. For example, within US-11 isolates, Curzate, Dithane, and Ridomil Gold all had a stronger effect on tomato than on potato isolates (Figures 1C and 1D). Comparison of the effects of each fungicide on the different groups of isolates also revealed interesting observations. For example, Curzate did not reduce infection by US-11 potato isolates (Figure 2A). On the other hand, it showed the highest inhibitory effect on US-11 tomato isolates. Considering all the tested isolates, Curzate seemed more effective on isolates collected from tomato than on those collected from potato (Figure 2A). Ridomil Gold had no effect on US-8 tomato isolates (Figure 2B). In addition, it even promoted infection caused by both US-11 and US-8 isolates from potato (Figure 2B). It reduced only infection caused by US-11 tomato isolates (Figure 2B). Although Acrobat slightly reduced infection by all groups of isolates, no differential responses were obtained (Figure 2C). Bravo reduced infection caused by all groups of isolates, with the best results on US-11 isolates from tomato (Figure 2D). This fungicide showed more inhibitory effects on US-8 than on US-11 isolates. Dithane either promoted the infection by P/US-11 isolates or had only a slight inhibition on the other groups (Figure 2E). Tattoo C reduced infection caused by all groups of isolates (Figure 2F).

Although the current study confirmed many results from the past *in vitro* tests (Daayf and Platt 2003), few differences were noted. For example, when Ridomil Gold was tested *in*

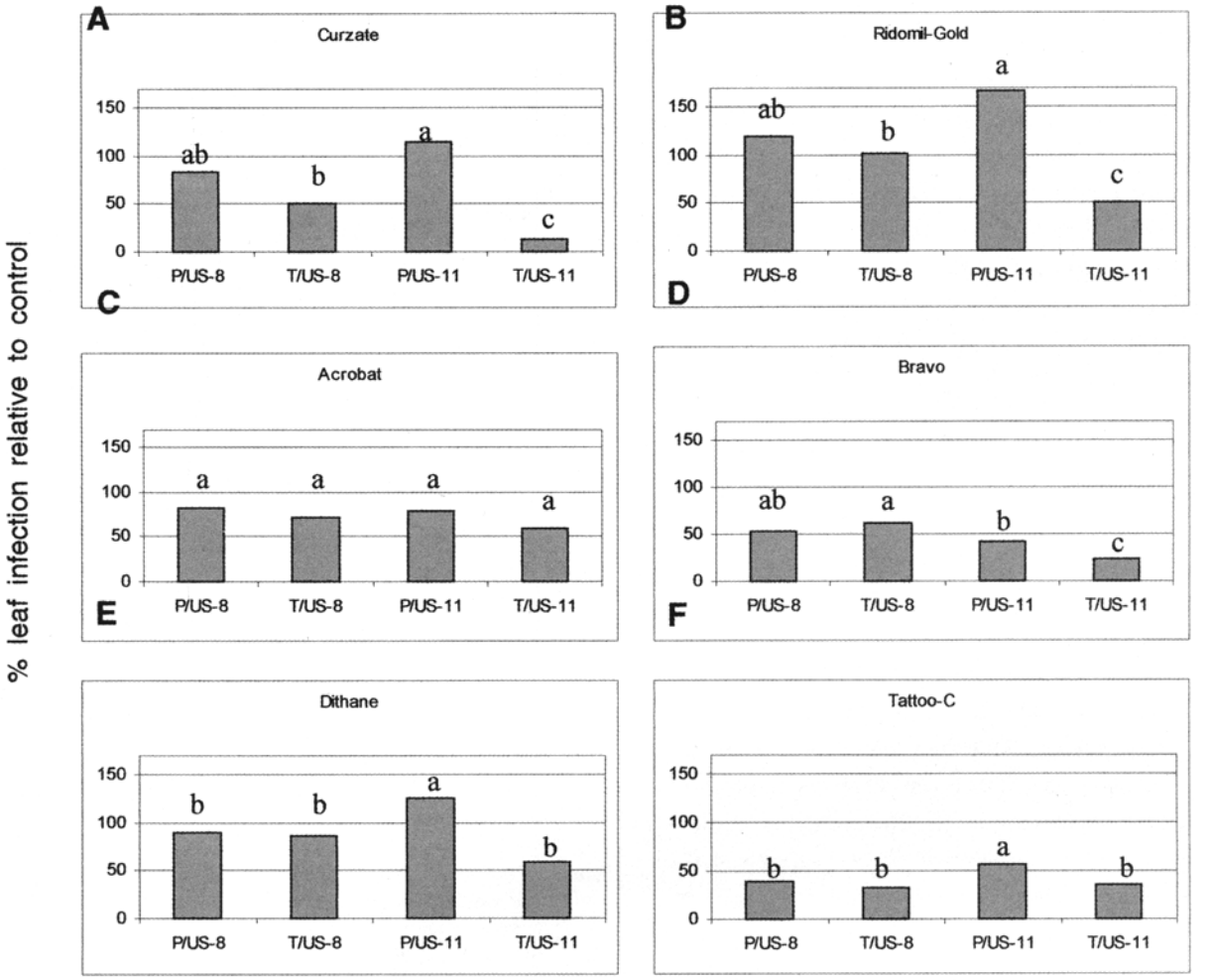

FIGURE 2.
Effects of each fungicide on late blight development in potato leaf discs caused by inoculation with P/US-8, P/US-11, T/US-8, and T/US-11 isolates of *P. infestans* (P = potato, T = tomato)

vitro, it provided a certain level of mycelial growth inhibition of US-8 isolates, whereas it did not have any effect on the same group of isolates on leaf discs. In addition, it reduced by 50% the infection caused by tomato/US-11 isolates while it promoted infection by potato/US-11. Another difference between the two tests referred to the used concentrations. Only concentrations below 10 µg a.i./mL were used *in vitro*, namely 1, 2.5, and 5 µg/mL, because at 10 µg a.i./mL, most fungicides were completely toxic to *P. infestans* isolates *in vitro* and no significant differences were observed. Actually it is likely that the concentrations tested in the two situations may not necessarily reflect the chemical concentration to which the pathogen is confronted in each situation. For example, while the concentrations used *in vitro* are easy to control, in the second type of tests leaf discs were simply dipped in a fungicide solution before being incubated, which makes it difficult to estimate the amount of fungicide that remains on the leaf surface. Given the smaller volume taken by the leaf discs in the latter situation, it is likely that the quantity of active ingredients available to the pathogen was lower with the leaf discs than during the *in vitro* tests, based on the same a.i. concentration. This shows how difficult it is to relate the concentra-

tions used in the field to those generally tested *in vitro*, and by consequence it reveals the validity of comparing equivalent a.i. concentrations among the tested fungicides.

Overall, *P. infestans* isolates from potato vs tomato showed different behaviors and management of late blight in these two important crops must take such differences into consideration. Information about genotypes present on each crop is crucial and might make a difference in terms of the nature and concentration of the fungicides to be applied. It is also important to keep in mind the over-time variability of *P. infestans* in terms of its genotypes and fitness as indicated by many reports in recent years (Chycoski and Punja 1996; Gisi and Cohen 1996; Koh et al. 1994). Such variability should be monitored as long as it is occurring and taken into account prior to making late blight management decisions.

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