Short Communication

Temporal Sensitivity of *Alternaria solani* to Foliar Fungicides

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ABSTRACT

Alternaria solani **Sorauer (Ellis) is a causal agent of early blight of potato (*Solanum tuberosum* L.). Sensitivity to chlorothalonil, triphenyl tin hydroxide (TPTH), and mancozeb was determined for field isolates *of A. solani* in 1998 and 1999. Specifically, the relative sensitivity of *A. solani* to foliar fungicides in field populations collected from several commercial fields was evaluated over the course of two growing seasons. Sensitivity to these foliar fungicides was determined with an *in vitro* spore-germination assay method. The fungicide concentration that inhibited conidial germination by 50% (EC$_{50}$) was estimated for each isolate. Sensitivity of *A. solani* isolates to TPTH and mancozeb varied little during the growing season, possibly because there were few TPTH and mancozeb applications made to the fields studied. At several locations, however, repeated exposure of *A. solani* populations to chlorothalonil resulted in considerable variability in sensitivity, frequently causing isolates to have decreased sensitivity to this fungicide at the end of the growing season. In five of seven fields, isolates of *A. solani* collected at the end of the season were significantly less sensitive to chlorothalonil than isolates collected at the beginning of the season.**

RESUMEN

Alternaria solani **Sorauer (Ellis) es el agente causal del tizón temprano de la papa (*Solanum tuberosum* L.). La sensibilidad al clorotalonil, trifenil hidroxido de estaño (TPTH) y mancozeb se determinó para aislamientos de campo de *A. solani* en 1998 y 1999. Específicamente se evaluó la sensibilidad relativa de *A. solani* a los fungicidas foliares en poblaciones de campo colectadas de varios campos comerciales en el transcurso de dos campañas de cultivo. La sensibilidad a estos fungicidas foliares fue determinada con un método *in vitro* de germinación de esporas. Para cada aislamiento se estimó la concentración de fungicida que inhibió la germinación de las conidias en un 50% (EC50). La sensibilidad de los aislamientos de *A. solani* al TPTH y al mancozeb variaron muy poco durante el período de cultivo, posiblemente porque se hicieron pocas aplicaciones de estos productos a los campos estudiados. En varios lugares, sin embargo, la repetida exposición de las poblaciones de *A. solani* al clorotalonil resultaron en una considerable variabilidad en sensibilidad, frecuentemente causando que los aislamientos disminuyan su sensibilidad a este fungicida al final de cada estación de cultivo. En cinco de siete campos, los aislamientos de *A. solani* colectados al final del cultivo fueron significativamente menos sensibles al clorotalonil que los aislamientos colectados al comienzo de la época de cultivo.**

INTRODUCTION

Early blight of potato, caused by *Alternaria solani* Sorauer (Ellis), poses a significant risk to crop productivity in the field and to tuber quality in storage (Nnodu et al. 1982). Early blight has re-emerged as an important disease in the Midwest, despite the increased use of fungicides for the management of late blight caused by *Phytophthora infestans* (Mont.) de Bary. A number of growers from Minnesota, Nebraska, and North Dakota reported a loss of early blight control near the end of the 1997 growing season that could not be abated through the

use of protectant fungicides applied as part of a normal foliar disease management program (Gudmestad, pers comm).

Early blight is primarily a disease of senescing tissue and is most severe on maturing or nutritionally deficient potato vines (Pscheidt and Stevenson 1988; Rotem 1981, 1994). It has been reported that severe epidemics can reduce yields by up to 30% (Christ and Maczuga 1989; Shtienberg et al. 1990). Current grower recommendations include a combination of cultural and chemical measures to prevent the development of early blight and to reduce the impact of this disease on yield. Although heritable resistance has been reported for *A. solani* (Christ 1991; Herriot et al. 1986, 1990; Holley et al. 1985), the disease is still primarily managed by the use of foliar fungicides (Easton and Nagle 1985; Harrison et al. 1965; Harrison and Venette 1970). Increased early blight severity, despite the widespread use of protectant fungicides, prompted us to study the sensitivity of *A. solani* to several protectant fungicides as a consequence of the increased number of applications required to manage late blight.

The annual occurrence of potato early blight necessitates repeated applications of fungicides to prevent significant loss of potato foliage. Fungicidal chemistries, such as chlorothalonil, mancozeb, triphenyl tin hydroxide, and azoxystrobin, are the most commonly used protectant and systemic fungicides used to combat early blight in the Midwest region. Chlorothalonil is a substituted benzene compound with broad-spectrum activity. Chlorothalonil, which has a multi-site mode of action, inhibits the formation of sulfur-containing enzymes (Sujkowski et al. 1995). The chemical class known as EBDCs (ethylenebisdithiocarbamates) include the fungicidal products mancozeb, maneb, and metiram. This preventative chemical class also has broad-spectrum activity and a multi-site mode of action. These chemicals break down to cyanide, which reacts with thiol compounds in the cell and interferes with sulfhydryl groups (Eckert 1988). This chemical class, as well as the substituted benzene compounds, is generally effective in controlling early blight. The risk of resistance development to these chemistries is generally considered low because they possess a multi-site mode of action (Georgopolus 1977). Chlorothalonil and mancozeb are two examples of protectant fungicides that have been used effectively for many years in late and early blight management without fungicide-resistance problems.

In contrast to EBDCs and substituted benzene compounds, triphenyl tin hydroxide (TPTH) possesses a limited spectrum of fungistatic activity, providing control of specific diseases such as early and late blight, scab, leaf blotch, and powdery mildew. Similar to other preventative fungicides, the mode of action involves several sites, which makes it difficult for fungi to develop resistance to the compound. Products included in this chemical group destroy cell membranes, thereby inhibiting the respiration process (Eckert 1988).

The overall purpose of this study was to determine if decreased sensitivity of *A. solani* isolates to foliar fungicides could be contributing to the current increase in the incidence and severity of early blight in the Midwest.

MATERIALS AND METHODS

Collection of A. solani *Isolates*

Leaf samples infected with the early blight fungus were collected in 1998 from two commercial fields (Fields 1 and 2) located near Kearney, NE, and two commercial fields (Fields 3 and 4) located near O'Neill, NE, (Table 1). These fields were on a 5- to 7-day fungicide spray schedule. They received eight to 12 applications of chlorothalonil at a rate of 1.02-1.24 kg a.i ha^{-1} (total chlorothalonil per season of 14.6-16.1 kg a.i. ha^{-1}) and three to five applications of a tank mix of chlorothalonil (0.88 kg a.i. ha^{-1}) combined with triphenyl tin hydroxide at a rate of 0.14 kg a.i. ha^{-1} (total TPTH applied 0.42-0.84 kg a.i. ha^{-1}) throughout the growing season. Each field was between 48.5 and 52.5 ha in size, and the early blight severity in these fields was relatively high with 30% to 50% defoliation. In addition, leaf samples were collected from Fields 5, 6, and 7, located near Park Rapids, MN, which also were on a 5- to 7-day spray schedule. These fields received nine to 11 applications of chlorothalonil at a rate of 0.88-1.02 kg a.i. ha^{-1} (total seasonal chlorothalonil of 10.1-12.0 kg a.i. ha^{-1}), alternating with three to five applications of a tank mix of mancozeb at a rate of 1.26 kg a.i. ha^{-1} (total mancozeb applied was 3.8-6.3 kg a.i. ha^{-1}) combined with triphenyl tin hydroxide at a rate of 0.14 kg a.i. ha^{-1} (total TPTH applied 0.42-0.70 kg a.i. ha^{-1}). These fields were approximately the same size as those previously described and early blight severity (20%-30%) was also relatively high in these fields.

Ten leaf samples were collected approximately biweekly (typically beginning in early July) throughout the 1998 growing season. Samples were collected randomly from infection foci near the center of each of the seven fields. Sample collection from the outer limits of the fields was avoided at all locations to minimize inter-field interference among *A. solani* populations.

TABLE 1—*Collection data on* Alternaria solani *isolates collected bi-weekly from fields in 1998 and 1999.*

Isolate Group[1]	Origin	Field	Cultivar	Collection date
ASJ1	Kearney, NE	Field 1	Pike	07/08/98
ASJ2	Kearney, NE	Field 1	Pike	07/16/98
ASJ3	Kearney, NE	Field 1	Pike	07/28/98
ASJ4	Kearney, NE	Field 1	Pike	08/28/98
ASN1	Kearney, NE	Field 2	Snowden	07/08/98
ASN2	Kearney, NE	Field 2	Snowden	07/16/98
ASN3	Kearney, NE	Field 2	Snowden	07/28/98
ASN4	Kearney, NE	Field 2	Snowden	08/28/98
ASSZ1	O'Neill, NE	Field 3	Russet Norkotah	07/09/98
ASSZ2	O'Neill, NE	Field 3	Russet Norkotah	07/23/98
ASSZ3	O'Neill, NE	Field 3	Russet Norkotah	07/29/98
ASL1	O'Neill, NE	Field 4	Russet Norkotah	07/09/98
ASL2	O'Neill, NE	Field 4	Russet Norkotah	07/23/98
ASL3	O'Neill, NE	Field 4	Russet Norkotah	07/29/98
ASL4	O'Neill, NE	Field 4	Russet Norkotah	08/28/98
ASPo1	Park Rapids, MN	Field 5	Russet Burbank	07/02/98
ASPo2	Park Rapids, MN	Field 5	Russet Burbank	07/24/98
ASPo3	Park Rapids, MN	Field 5	Russet Burbank	08/13/98
ASPo4	Park Rapids, MN	Field 5	Russet Burbank	09/04/98
ASB1	Park Rapids, MN	Field 6	Russet Burbank	07/02/98
ASB2	Park Rapids, MN	Field 6	Russet Burbank	07/24/98
ASB3	Park Rapids, MN	Field 6	Russet Burbank	08/13/98
ASB4	Park Rapids, MN	Field 6	Russet Burbank	09/04/98
AP98-1	Park Rapids, MN	Field 7	Russet Burbank	07/02/98
AP98-2	Park Rapids, MN	Field 7	Russet Burbank	07/24/98
AP98-3	Park Rapids, MN	Field 7	Russet Burbank	08/13/98
AP98-4	Park Rapids, MN	Field 7	Russet Burbank	09/04/98
AP99-1	Park Rapids, MN	Field 7	Russet Burbank	07/14/99
AP99-2	Park Rapids, MN	Field 7	Russet Burbank	07/28/99
AP99-3	Park Rapids, MN	Field 7	Russet Burbank	08/11/99
AP99-4	Park Rapids, MN	Field 7	Russet Burbank	08/25/99

[1]Isolates were recovered from 10 potato leaf samples (two source isolates per leaf) for a total of 20 isolates per isolate grouping. Isolates are grouped by field and sampling date; for example, ASJ1, AS = *A. solani*; J = field designation; 1= first sampling date.

Seasonal sensitivity of *A. solani* to fungicides was studied again in 1999. The O'Neill, NE, and Park Rapids, MN, locations were chosen for the second year of study based on results obtained in 1998. Four 50-foot rows of 'Russet Burbank' tubers were planted in Field #7 (Park Rapids, MN). Field #7 was chosen at Park Rapids, MN, because it was the most accessible and amenable for conducting a small plot in 1999. The potato plot was planted near the center of Field #7 while the rest of the field was planted to corn. These rows received eight applications of chlorothalonil, at a rate of 0.88 kg a.i. ha^{-1}, throughout the growing season and one application of a tank mix of chlorothalonil (0.88 kg a.i. ha^{-1}), combined with triphenyl tin hydroxide (0.14 kg a.i. ha^{-1}) near the end of the growing season. Ten leaf samples were randomly collected on a biweekly basis,

and *in vitro* fungicide sensitivity tests were conducted in order to compare the 1998 population to the 1999 population within the same field. Field #3 near O'Neill, NE, also was chosen for a small plot study in 1999, but was lost due to a misapplication of herbicide.

Isolation and Spore Production

Isolates were recovered from sections of plant material with early blight lesions. The sections were surface-sterilized in a 1% sodium hypochlorite solution for 1 min, rinsed in sterile distilled water, placed on water agar or acidified potato-dextrose agar (PDA), and incubated under continuous light at 20 ± 1 C. Mycelia growing from the diseased tissue were hyphal-tipped and transferred onto plates containing 10 mL of clarified V-8 juice agar ([CV-8], Campbell's® V-8 juice, 100 mL; $CaCO_3$, 1.5 g; agar, 15 g; and distilled water, 900 mL). Hyphal tip sections were grown for 7 days (23-25 C) on CV-8 medium under near-ultraviolet light (310-400 nm) radiation from one 33-W black light fluorescent lamp and two 33-W cool-white fluorescent lights with a 16-h photoperiod.

Single spores from hyphal-tipped fungal colonies were transferred onto plates containing CV-8 agar in order to obtain pure cultures of *A. solani*. Single-spore isolates were incubated for 3 days (23-25 C) under 33-W black light fluorescent and cool-white fluorescent lights to induce sporulation. The isolates were maintained on CV-8 agar medium at 20 ± 1 C for 7 days and then maintained at –80 C for long-term storage. Original leaves were preserved as dried, herbarium-type specimens.

A baseline isolate was included in the study for comparison. Isolate ATCC1 was obtained from the American Type Culture Collection (#32904) and had been isolated from tomato infected with *A. solani* in 1974.

In vitro Assessment of Fungicide Sensitivity

Fungicide sensitivity was determined by comparing the spore germination of each isolate on fungicide-amended water agar with the spore germination on unamended agar medium. Water agar was amended with the following fungicides as formulated commercial products dissolved in 0.1% dimethyl sulfoxide (DMSO): chlorothalonil (Bravo Ultrex; Zeneca Ag. Products, Richmond, CA), triphenyl tin hydroxide (Super Tin, Griffin Co., Valdosta, GA), and mancozeb (Dithane DF, Rohm and Haas, Philadephia, PA). The final concentrations of chlorothalonil, triphenyl tin hydroxide, and mancozeb in the media were 0, 0.01, 0.10, 1.0, 10.0, 100.0, and 1000.0 a.i.

mg mL^{-1}. The control was amended with DMSO (0.1%) without fungicide.

After conidia were produced on the CV-8 plates (approximately 12-14 days), sterile, distilled water containing five drops of Tween 20/100 mL was added to the plates, and the conidia were dislodged from the agar surface. The concentration of the conidial suspension was determined with a hemacytometer. After 500 mL of the conidial suspension at 10^4 conidia mL^{-1} had been randomly added to the control plate and plates containing fungicide-amended medium, the conidial suspension was spread evenly across each plate using a sterile glass rod and allowed to dry in a laminar flow hood. The plates were placed in random arrangement in a humidity chamber (Conviron" CWP 3023) approximately 1 m from the light source under continuous light at 20 ± 1 C for 24 h. The germination of 100 conidia then was evaluated. A conidium was considered germinated if a single germ tube was equal in length to the conidium or if multiple cells produced germ tubes approximately equal in length to each other.

The fungicide-sensitivity experiments were set in a completely randomized design. For each collection date in each field, single spore isolates from 10 leaves (two separate source isolates per leaf) for a total of 20 isolates were tested for fungicide sensitivity. Each experiment was performed twice with two replications per concentration for each fungicide/isolate combination. For the purposes of data presentation, isolates were grouped by field and sampling date (Table 1).

Statistical Analyses

EC$_{50}$ (effective concentration at which the spore germination is 50% of control) values were determined using data from the spore-germination assay. Data obtained from the spore-germination assay was transformed into percentage of reduction and plotted against the logarithm of the chemical concentration. Following Goldstein's (1964) procedure for non-quantal bioassays to estimate EC$_{50}$, a regression line was used to estimate the fungicide concentration at which growth was reduced by 50%.

For the spore-germination assay, each independent experiment was analyzed separately, and Bartlett's Chi-Square Test was used to test for homogeneity of variance between the two independent experiments. The combined data obtained from the germinated spore assay were analyzed using analysis of variance and EC$_{50}$ means were separated by Duncan's Multiple Range Test. The ANOVA was performed on EC$_{50}$ values obtained from the germinated spore assay by using the Statis-

tical Analysis System (SAS Institute, Inc., Raleigh, NC). A significant value of $P = 0.05$ was used in all statistical tests.

RESULTS AND DISCUSSION

Field populations of A. solani varied little in their sensitivity to TPTH (Figure 1) and mancozeb (Figure 2) over the course of a growing season. Sensitivity of A. solani populations to TPTH were generally not significantly different between the collection dates (Figures 1B,C,E,G). Most of the isolates had approximately the same EC$_{50}$ value in response to TPTH ($\approx$3.00 mg mL^{-1}), although the baseline isolate (ATCC1) had a lower EC$_{50}$ value (0.33 mg mL^{-1}) compared to the other isolates included in this study. Variability did exist between A. solani isolates in their sensitivity to TPTH in one field located in Kearney, NE, (Field 1) (Figure 1A). Isolates of A. solani collected in this field during the last two collection dates were significantly less sensitive to TPTH than isolates collected at the beginning of the season. The EC$_{50}$ value for isolate groups ASJ1 and ASJ2 was approximately 3.00 mg mL^{-1}; in contrast, the EC$_{50}$ value for isolate group ASJ3 was 4.90 mg mL^{-1} while isolate group ASJ4 had an EC$_{50}$ value of approximately 4.00 mg mL^{-1} (Figure 1A). Differences between TPTH EC$_{50}$ values for the isolates recovered from Fields 6 and 7 (Figures 1F and 1H) were also significantly different from each other ($P = 0.05$); however, these differences were small and not associated with a loss of sensitivity to TPTH at the end of the season.

Mancozeb EC$_{50}$ values for the isolates tested ranged from 0.50-3.00 mg mL^{-1} (Figures 2A-H). The baseline isolate (ATCC1) was sensitive to mancozeb with an EC$_{50}$ value of 0.71 mg mL^{-1}. Mancozeb EC$_{50}$ values for A. solani isolates differed significantly ($P = 0.05$) at all of the locations. Although this may be due to inherent variability in sensitivity of A. solani to this fungicide, it is interesting to note that in three fields, isolates collected at the end of the season were more sensitive to mancozeb than those collected at the beginning (Figure 2E, F, H). However, isolate group ASJ4, collected at the end of the growing season in Field #1 was significantly less sensitive than isolates collected earlier in the collection period (Figure 2A).

Significant variability in sensitivity to chlorothalonil existed between the A. solani isolates collected in 1998. Results indicated that there were significant differences ($P = 0.05$) between the chlorothalonil EC$_{50}$ values for the A. solani isolates in five of the seven locations in 1998. A. solani tended to have reduced sensitivity to chlorothalonil at the end of the collection period as observed in one field located near Kearney,
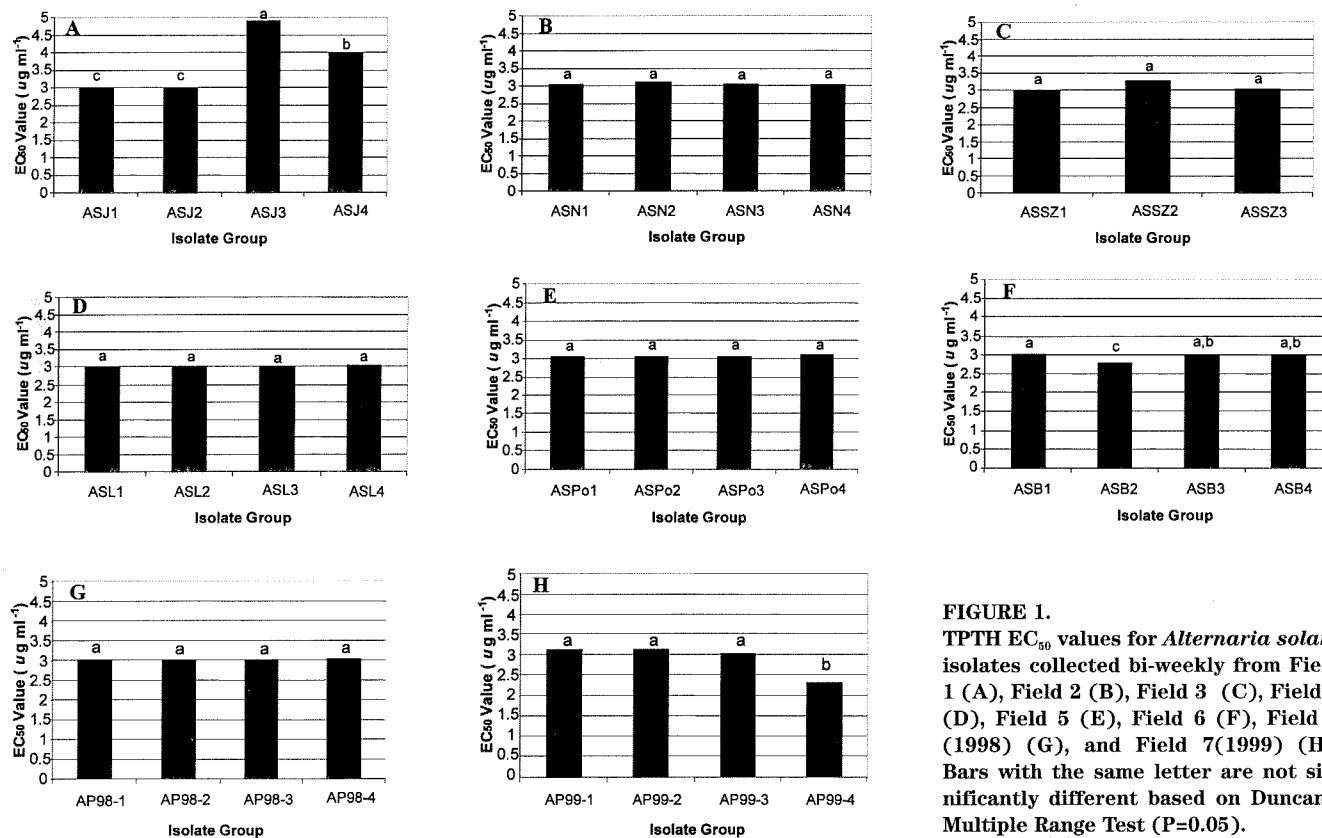
FIGURE 1.

TPTH EC_{50} values for *Alternaria solani* isolates collected bi-weekly from Field 1 (A), Field 2 (B), Field 3 (C), Field 4 (D), Field 5 (E), Field 6 (F), Field 7 (1998) (G), and Field 7(1999) (H). Bars with the same letter are not significantly different based on Duncan's Multiple Range Test (P=0.05).
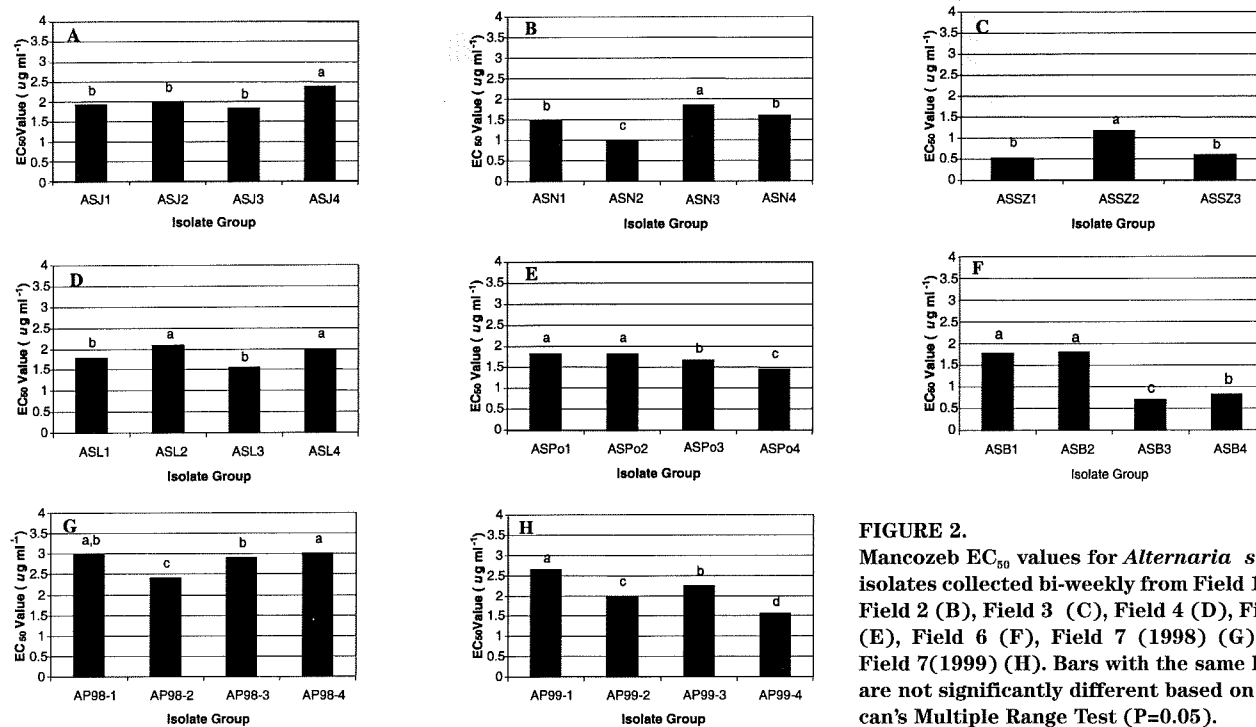
FIGURE 2.

Mancozeb EC_{50} values for *Alternaria solani* isolates collected bi-weekly from Field 1 (A), Field 2 (B), Field 3 (C), Field 4 (D), Field 5 (E), Field 6 (F), Field 7 (1998) (G), and Field 7(1999) (H). Bars with the same letter are not significantly different based on Duncan's Multiple Range Test (P=0.05).
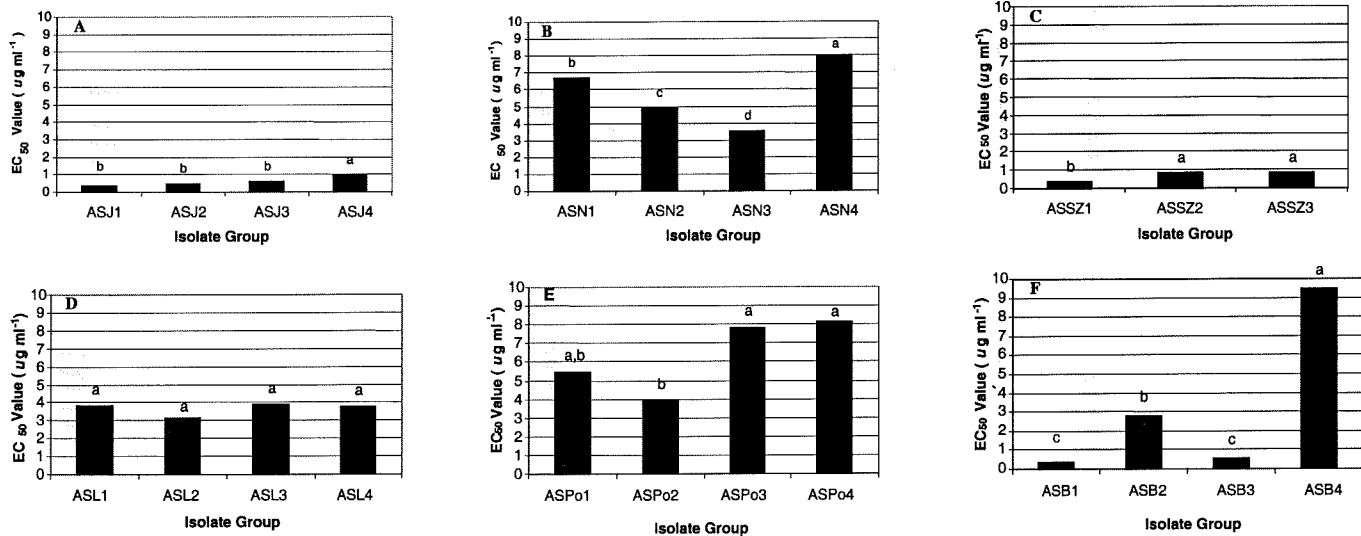
FIGURE 3.
Chlorothalonil EC$_{50}$ values for *Alternaria solani* isolates collected bi-weekly from Field 1 (A), Field 2 (B), Field 3 (C), Field 4 (D), Field 5 (E) and Field 6 (F). Bars with the same letter are not significantly different based on Duncan's Multiple Range Test (P=0.05).
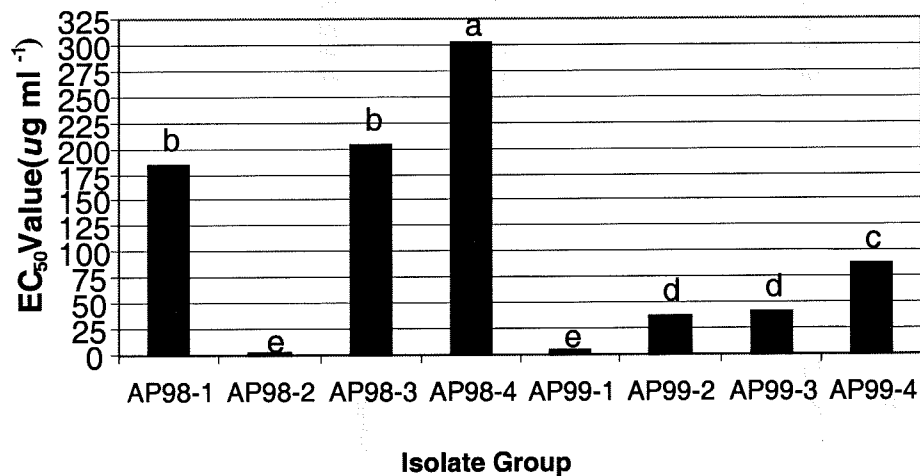

FIGURE 4.
Chlorothalonil EC$_{50}$ values for *Alternaria solani* isolates collected bi-weekly from Field 7 in 1998 and 1999. Bars with the same letter are not significantly different based on Duncan's Multiple Range Test (P=0.05).

NE, (Field 1) (Figure 3A), one field located near O'Neill, NE, (Field 3) (Figure 3C) and in three fields located near Park Rapids, MN, (Fields 5, 6, and 7) (Figures 3E, F, and Figure 4). Isolates of *A. solani* obtained from the last collection date in these fields were significantly less sensitive to chlorothalonil than isolates collected from the first collection date. However, in contrast to those field populations, isolates recovered from a field near Kearney, NE, (Field 2) (Figure 3B) and another field located near O'Neill, NE, (Field 4) (Figure 3D) demonstrated no significant decrease in sensitivity to chlorothalonil

over the collection period. The baseline isolate (ATCC1) included in this study was sensitive to chlorothalonil with an EC$_{50}$ value of 0.70 mg ml^{-1}.

Regular applications of chlorothalonil in 1999 resulted in reduced sensitivity of the *A. solani* population present within Field 7 near Park Rapids, MN, (Figure 4) similar to what was observed in this field in 1998. The chlorothalonil EC$_{50}$ values for isolate groups AP99-1, AP99-2, AP99-3, and AP99-4 were 4.08, 37.00, 40.00, and 86.25 mg mL^{-1}, respectively. The EC$_{50}$ value for isolate group AP99-4, collected near the end of the growing

season, was significantly higher than the other three isolate groups collected that year. The EC_{50} value for AP99-1 collected on 14 July 1999, was 4.08 mg mL^{-1}, which was significantly lower than the EC_{50} value for AP98-4 (300.0 mg mL^{-1}) collected at the end of the 1998 growing season. In addition, the EC_{50} values for *A. solani* isolates collected at the beginning of the 1998 and 1999 growing seasons were similar. In 1998, AP98-2 (collection date = 7/24/98) had an EC_{50} value of 7.0 mg mL^{-1}, which is slightly higher, but not significantly, than the EC_{50} value (4.08 mg mL^{-1}) for AP99-1 (collection date = 7/13/99).

Resistance and reduced sensitivity to fungicides among fungal plant pathogens are significant problems in the area of chemical disease management. Management of potato early blight requires effective monitoring of fungicide resistance in *A. solani* populations. Due to the increased severity of early blight in 1997-1998 in many areas of the United States, particularly in the Midwest, the primary objective of this study was to determine if variability existed in the sensitivity of *A. solani* field populations to protectant fungicides. We hypothesized that *A. solani* populations may become less sensitive to a specific fungicide following repeated applications and that this reduced sensitivity may contribute to a loss of disease control.

A key component to a monitoring program is to utilize an assay method (*in vivo* or *in vitro*) that will accurately measure the sensitivity of the particular pathogen to different fungicide chemistries. Preliminary work conducted in our laboratory determined that differences between a spore-germination and a radial-growth assay method were significant when comparing the EC_{50} values, similar to previously published studies (Lorenz 1988). The sensitivity of the *A. solani* isolates included in this study to various fungicide chemistries was described using the results obtained from the spore-germination assay method. Because preventative fungicides (i.e., chlorothalonil, mancozeb, and triphenyl tin hydroxide) provide a chemical barrier to the pathogen (preventing the establishment of the pathogen), it was felt that the spore-germination assay would more accurately assess the sensitivity of *A. solani* isolates to these foliar fungicides.

There are several examples of pathogen populations that express reduced sensitivity to fungicides under the selection pressure from repeated fungicide use (Eckert 1988). For example, reduced sensitivity of powdery mildew to demethylation-inhibiting fungicides has been reported (Brent 1988; Hearney 1988). Results obtained from this study indicate that there were differences in the degree of sensitivity to chlorothalonil within several *A. solani* field populations. In a number of fields sampled, there appeared to be a temporal shift in sensitivity of *A. solani* to chlorothalonil over the course of the growing season. For several of the fields sampled in 1998 (five of seven), isolates collected at the end of the growing season had significantly higher EC_{50} values than isolates collected at the beginning of the growing season. However, not all of the *A. solani* field populations expressed this change in sensitivity during the growing season. Interestingly, the three fields in Minnesota (Fields 5, 6, 7) where *A. solani* populations at the end of the growing season were significantly less sensitive to chlorothalonil than populations at the beginning of the season were also those fields that had the lowest total amount of the fungicide applied during the season (10.1-12.0 kg a.i. ha^{-1}). These three fields also had lower chlorothalonil dose rates applied (0.88-1.02 kg a.i. ha^{-1}) than other fields sampled in this study. In contrast, the two fields in Nebraska (Fields 2 and 4) where sensitivity to chlorothalonil did not change within the *A. solani* population were also the fields that had the highest total amount of this fungicide applied during the season (15.3-16.1 kg a.i. ha^{-1}) and had the highest chlorothalonil dose rates at each application (1.02-1.24 kg a.i. ha^{-1}). Whether the total amount of fungicide to which the fungal population is exposed or the application dose rate has an effect on reduced sensitivity is not known at this time. We do know, however, that the lowest label dose rate for chlorothalonil (0.88 kg a.i. ha^{-1}) that we used in 1999 in Field #7 also induced a temporal shift in sensitivity to this fungicide (Figure 4). Differences in cultivar susceptibility, fungicide use and rate, as well as differences in environment, may also interact with fungicide application to exert moderating influences in fungicide sensitivity within an *A. solani* population.

A temporal change in sensitivity to chlorothalonil was observed in the *A. solani* population present within Field 7 in 1998 and 1999. Because the *A. solani* population present within Field 7 at the beginning of the 1999 growing season was more sensitive to chlorothalonil than the *A. solani* population present within this field at the end of the 1998 growing season, we conclude that the decreased sensitivity observed in this field cannot be considered resistance. We believe that the temporal changes in sensitivity in Field 7 may have occurred due to the increased inputs of chlorothalonil applied at a relatively low label rate (0.88 kg a.i./ha) and once the chlorothalonil applications ceased, the *A. solani* population appeared to revert to being sensitive to the fungicide. As a result, we

describe what was observed in these fields as decreased sensitivity because we did not observe a stable, heritable resistance within the *A. solani* population (Dekker 1993). In addition, because chlorothalonil is a multi-site inhibitor, the development of resistance to this chemical class is low. *In vivo* tests need to be conducted to determine if the decreased chlorothalonil sensitivity in *A. solani* isolates collected at the end of the 1998 growing season would equate to a significant loss in disease control compared to isolates collected at the beginning of 1999 growing season.

The decreased sensitivity in response to chlorothalonil appears to be fungicide specific. The isolates that expressed high chlorothalonil EC_{50} values did not express decreased sensitivity to TPTH or mancozeb. Furthermore, the fact that the *A. solani* populations we sampled did not change appreciably in their sensitivity to TPTH or mancozeb over the course of the growing season, further corroborates our observation that these populations did become significantly less sensitive to chlorothalonil during the growing season due to repeated exposure to this specific fungicide.

There have been no previous reports of shifts of sensitivity to chlorothalonil in *A. solani*. Sujkowski et al. (1995) found decreased sensitivity to chlorothalonil among Mexican isolates of *Phytophthora infestans*. Further studies would be required to determine the mechanism responsible for this temporary decrease in sensitivity to chlorothalonil in *A. solani*. Decreased sensitivity and resistance to fungicides with a multi-site mode of action may be due to mechanisms such as detoxification, reduced uptake, or increased efflux of the fungicide (Sisler 1988). If results from future studies concur with those obtained here and also determine that *A. solani* can become seasonally insensitive to fungicides such as chlorothalonil when sub-lethal doses of this fungicides are used, recommendations for control of early blight caused by *A. solani* may include a fungicide program including chlorothalonil at the high rate (1.24 kg a.i./ha) alternating with another foliar fungicide (e.g., azoxystrobin, TPTH, or mancozeb).

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