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Sensitivity of Jordanian isolates of *Alternaria solani* to mancothane

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Summary. Early blight of potato, caused by *Alternaria solani*, poses a significant risk to potato crops worldwide. Fifty *A. solani* isolates representing a population were collected from the Jordan Valley, purified, and tested for their sensitivity to the fungicide mancothane. The isolates were tested against a series of concentrations of 0, 0.1, 1, 10, 100, and 1000 mg mancothane ml^{-1} in 5% sodium dodecyl sulfate (SDS). Some *A. solani* isolates tolerated up to 1000 mg mancothane ml^{-1}. Isolates treated with the various concentrations were divided into 5 groups based on the percentage of *A. solani* growth achieved despite treatment: group 1 comprised isolates with mycelial growth of 0.1–20.9%; group 2, 21–40.9%; group 3, 41–60.9%; group 4, 61–80.9%; and group 5, 81–100%. Ninety-seven percent of all isolates grew at 0.1 mg mancothane ml^{-1}, 94% at 1 mg ml^{-1}, 86% at 10 mg ml^{-1}, 66% at 100 mg ml^{-1}, and 16% at 1000 mg ml^{-1}. Isolates appeared to be distributed normally at 10 mg mancothane ml^{-1}. Eight isolates were highly resistant to mancothane and grew even at the highest test concentration. An *A. solani* population collected from potato fields in the Jordan Valley exhibited a moderate level of resistance to mancothane. Growers should be careful and vigilant when using this fungicide to control early blight.

Key words: early blight, ethylene bisdithiocarbamate, mancothane, mancozeb, potato.

Introduction

Alternaria solani, the cause of early blight of potato (*Solanum tuberosum* L.), is becoming a serious problem in the potato industry of many countries (Nnodu *et al*., 1982; Christ and Maczuga, 1989; Shtienberg *et al*., 1990). Early blight poses a significant risk to crop productivity in the field and to tuber quality in storage. In some areas, severe epidemics have caused storage losses of up to 30% (Nnodu *et al*., 1982). Studies have shown that tuber infection occurs at harvest, and decay develops in storage. The reason for this is thought to be that spores present on the soil surface and on the potato foliage infect the tubers through wounds created during harvesting operations. If this is so, surface disinfection of freshly harvested tubers should be able to reduce or eliminate infection in storage (Harrison and Franc, 1988).

Early blight is most severe on maturing or nutritionally deficient potato vines (Rotem, 1981, 1994; Pscheidt and Stevenson, 1988). Disease control recommendations are currently based on an integrated pest management approach, which comprises a combination of cultural and chemical practices to prevent the development of early blight and reduce its impact on yield. Despite the fact that resistance to *A. solani* has been reported (Herriot *et al*., 1986, 1990; Christ, 1991; Holley *et al*., 1995),

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the disease is still primarily managed by foliar fungicides (Harrison *et al*., 1965; Harrison and Venette, 1970; Easton and Nagle, 1985). As early blight occurs annually in potato fields, repeated applications of fungicides to prevent significant loss of potato foliage are necessary. Fungicides such as chlorothalonil, mancozeb, triphenyl tin hydroxide and azoxystrobin are some of the most commonly used protectant and systemic fungicides used to combat early blight throughout the world. The chemical class of the ethylene bisdithiocarbamates (EBDCs) includes the preventative fungicides mancozeb, maneb, and metiram. This class, which has broad-spectrum activity and a multi-site mode of action, is generally effective in controlling early blight, and its constituent chemicals break down to cyanide, which reacts with thiol compounds in the cell and interferes with sulfhydryl groups (Eckert, 1988). Because of their multi-site mode of action the risk of plants becoming resistant to these fungicides is generally considered low (Georgopolus, 1977). Among the protective fungicides, chlorothalonil and mancozeb have been used effectively for many years in controlling late and early blight without fungicide resistance problems.

Mancothane (manganese ethylene bisdithiocarbamate complex with zinc salt) also possesses a multi-site mode-of-action, and inhibits enzyme activity in fungi by forming a chemical complex with metal-containing enzymes, including those involved in the production of ATP. Mancothane, which is also known as mancozeb, tatodust, dithane M-45, and manzate, is rapidly degraded in the environment by hydrolysis, oxidation, photolysis, and plant metabolism (Tomlin, 2000).

High levels of early blight in Jordan despite the widespread use of protectant fungicides suggested to study the sensitivity of the local population of *A. solani* to mancothane (mancozeb), the fungicide commonly used to control early blight in Jordan.

Materials and methods

Sample collection

Potato leaf samples infected with the early blight fungus were collected in 1999 and 2000 from commercial potato fields in the Jordan Valley. Ten leaf samples were collected at 2-wk intervals throughout each growing season. Samples were collected randomly from infection foci near the center of each field. Sample collection from the outer limits of the fields was avoided to minimize inter-field interference among *A. solani* populations.

Alternaria solani isolation and spore production

Fifty isolates of *A. solani* were recovered from sections of potato leaves with early-blight lesions. The leaf-sections were surface sterilized in a 1% sodium hypochlorite solution for 1 min, rinsed with sterile distilled water, placed on potato dextrose agar (PDA), and incubated under continuous light at 20±1°C. Mycelium that grew from diseased tissue was hyphal-tipped and transferred to Petri dishes each containing 15 ml PDA. Hyphal-tip sections were grown for 7 days at 23–25°C on PDA under near-ultraviolet light (310–400 nm) with a 16-h day.

Single spores from hyphal-tipped fungal colonies were transferred to PDA dishes to obtain pure cultures of *A. solani*. Single-spore isolates were incubated for 3 days at 23–25°C under fluorescent black light and cool-white light to induce sporulation. The isolates were maintained on PDA at 20±1°C for 7 days.

In vitro assessment of fungal sensitivity to mancothane

Sensitivity of isolates to mancothane (mancozeb) was determined by comparing spore germination of each of the fifty isolates on fungicide-amended PDA with spore germination on unamended PDA. Mancothane 80% WP was kindly provided by the Veterinary and Agricultural Products Manufacturing Co. Ltd. (VAPCO), Amman, Jordan. Mancothane was dissolved in 5% sodium dodecyl sulfate (SDS) and was brought to final concentrations of 0, 0.1, 1, 10, 100, and 1000 mg a.i. ml^{-1}.

Prior to adding the fungicide, PDA medium was amended with 30 mg l^{-1} streptomycin to suppress bacterial growth. Mancothane (2 ml) at the appropriate concentration was then added to the PDA dishes and left for 4 hours before *A. solani* cultures were transferred to the dishes. Control dishes received 2 ml of 5% SDS each.

With a 10-cm-long spring-loaded plunger of 5 mm diameter, a plug of inoculum from the actively growing margin of a Petri dish culture of each fungal isolate was placed at the center of each Petri dish with the mycelium face down. Each isolate was inoculated on eight plates and incubated for 7 days at room temperature (ca. 22°C). The experi-

ment was performed twice, with eight replications per concentration for each isolate.

Seven days after inoculation, the radial growth of two early-blight colonies on PDA dishes, 90° apart, was measured. Percent fungal growth due to mancothane was calculated as follows: % colony growth = (T/C) × 100, where T is the mean colony diameter of each fungus; and C is the mean colony diameter of the control. Values calculated for the various treatments were expressed as a percentage of the control. Percent fungal colony growth in each trial was the mean of eight determinations. The mean values of two trials were calculated and are reported.

Percent growth values for each isolate at each fungicide concentration were divided into 5 groups on the basis of colony growth achieved as a percentage of control colony growth (=growth at 0 mg mancothane/ml^{-1} SDS). Group 1 isolates had fungal colony growth between 0.1 and 20.9% of control growth; colonies in group 2 were between 21 and 40.9% of control growth; in group 3 between 41 and 60.9%; in group 4 between 61 and 80.9%; and in group 5 between 81 and 100%. The number of isolates in each group at each concentration of mancothane is shown in the graphs.

Results and discussion

Field populations of *A. solani* varied in their sensitivity to mancothane (Fig. 1). Ninety-seven percent of isolates grew with a concentration of 0.1 mg mancothane ml^{-1} SDS, 94% with 1 mg ml^{-1}, 86% with 10 mg ml^{-1}, 66% with 100 mg ml^{-1}, and 16% with 1000 mg ml^{-1} (Fig. 2). Isolates were distributed normally at 10 mg ml^{-1}. At 0.1 mg mancothane ml^{-1}, isolates had growth between 41 and 100%. The majority of isolates (62%) at this concentration had growth between 81 and 100%; 26% of isolates had growth between 61 and 80.99%; and 8% growth between 41 and 60.9%. Two isolates were completely inhibited at this concentration. At 1.0 mg mancothane ml^{-1}, the majority of early-blight isolates within the population (44%) fell in the middle range with 61–80.9% colony growth. Fourteen, 24 and 12% of isolates grew between 21 and 40.9, 41 and 60.9, and 81 and 100% respectively. Three isolates were completely inhibited at this concentration. At 10 mg mancothane ml^{-1}, the growth of 7 isolates was completely inhibited, and none of the isolates exceeded 80% of control colony growth. The remaining isolates fell in between; the highest percentage of isolates (30%) had colony growth between 41 and 60.9%, followed by 28% of isolates with growth between 21 and 40%, and 22% of isolates with growth between 61 and 80.9%. At 100 mg ml^{-1}, colony growth of 34% of isolates was completely inhibited and none of the isolates exceeded 70% of control colony growth. Twenty percent of isolates had colony growth between 21 and 40.9% and an equal percentage between 41 and 60.9%. Twelve isolates had colony growth between 0.1 and 20.9%. Only one isolate achieved 61–80.9% colony growth. At the highest mancothane concentration of 1000 mg ml^{-1}, none of the isolates within the population exceeded colony growth of 32%, and most isolates (84%) were completely inhibited. Only 5 isolates (10%) produced colony growth between 0.1 and 20%, and three isolates (6%) had 21–40.9% colony growth (Fig. 1). Average percent growth of all 50 isolates, including minimum and maximum growth, is shown in Table 1. Two isolates were completely inhibited at 0.1 mg ml^{-1}, 3 at 1 mg ml^{-1}, 7 at 10 mg ml^{-1}, 17 at 100 mg ml^{-1}, and 42 at 1000 mg ml^{-1}.

Regular applications of mancothane reduced the sensitivity of the *A. solani* population to the fungicide. Both resistance and reduced sensitivity of plant pathogens to fungicides are significant problems in the area of chemical disease management. The control of early blight requires careful monitoring of fungicide resistance in the *A. solani* populations. Because of the increasing severity of early blight in many parts of Jordan, the primary objective of this study was to determine whether the *A. solani* field population varied in its sensitivity to the protectant fungicide mancothane. It is hypothesized that this population becomes less sensitive to a mancothane after repeated applications

Table 1. Percent growth of 50 isolates of *Alternaria solani* subjected *in vitro* to various concentrations of mancothane.

Mancothane concentration (mg ml^{-1})	Percent growth		
	Minimum	Maximum	Average ± S.E.
0.1	0.0	99.8	81.7 ± 1.8
1	0.0	90.9	59.0 ± 1.1
10	0.0	75.3	39.6 ± 0.9
100	0.0	69.8	22.1 ± 0.7
1000	0.0	32.0	2.8 ± 0.1

and that the reduced sensitivity may impair the disease control provided by the fungicide.

Preventive fungicides such as mancothane create a chemical barrier to the pathogen, preventing its establishment. However, there are several cases of pathogen populations whose sensitivity to fungicides was reduced as consequence of selection pressure from repeated fungicide use (Eckert, 1988; Al-Mughrabi and Gray, 1995a). An example is the lowered sensitivity of powdery mildew to demethylation-inhibiting fungicides (Brent, 1988; Hearney, 1988; Al-Mughrabi and Gray, 1995b). The present

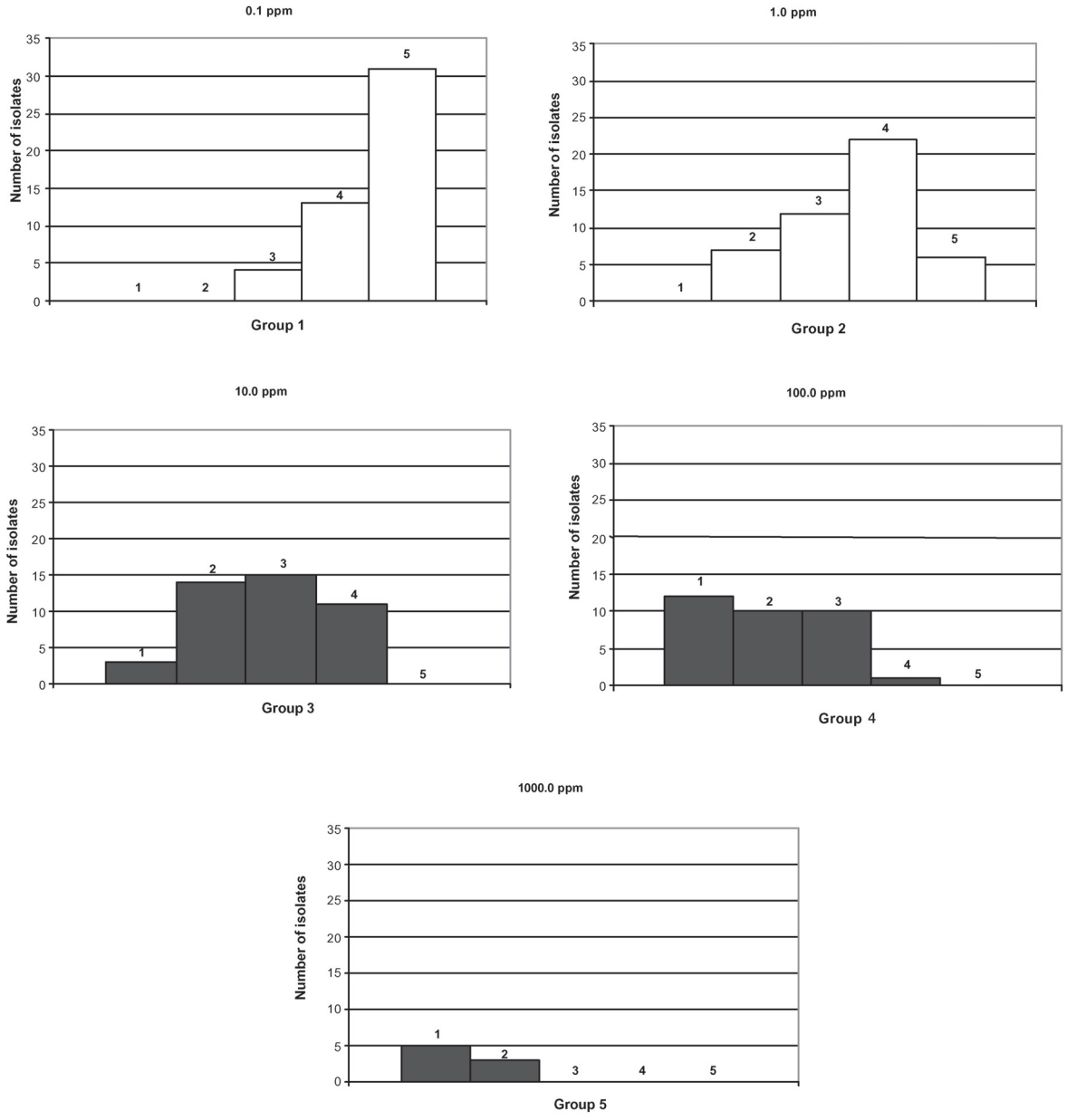

Fig. 1. Isolates of *Alternaria solani* classed into 5 groups based on percent growth *in vitro* with various concentrations of mancothane. Group 1 includes all isolates with colony growth between 0.1 and 20.9%; group 2, 21.0–40.9%; group 3, 41–60.9%; group 4, 61–80.9%; and group 5, 81–100%.

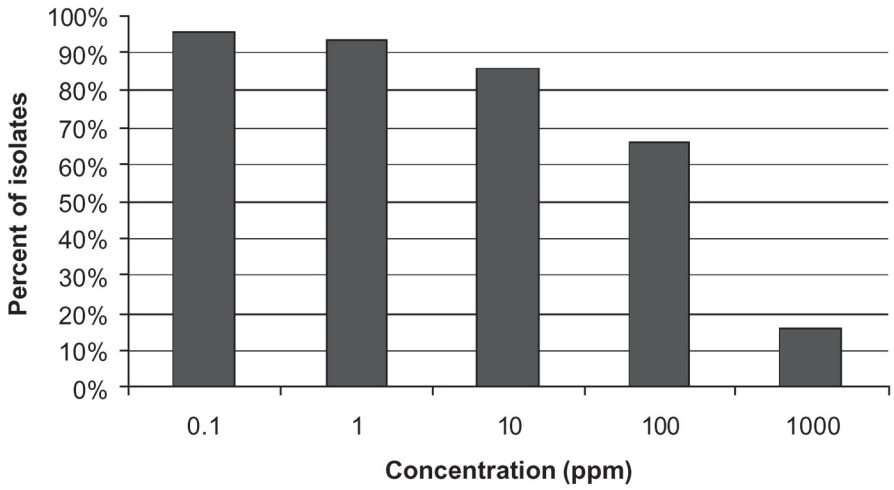

Fig. 2. Percent of isolates of *Alternaria solani* that grew *in vitro* at various concentrations of mancothane.

study showed that sensitivity to mancothane varied within the *A. solani* population from the Jordan Valley. Whether the total amount of mancothane to which the fungal population is exposed or its application rate has an effect on lowering sensitivity is not known at present. Differences in cultivar susceptibility, in fungicide use and application rate, and in the environment, may also have an effect on mancothane in the field, moderating sensitivity to it in the *A. solani* population. Mancothane degrades rapidly in the environment by hydrolysis, oxidation, photolysis, and plant metabolism (Tomlin, 2000). The main water source for potato growers in the Jordan Valley is the King Abdullah Canal (Al-Mughrabi and Nazer, 1991). This water has a rather high pH values ranging from 7.3 to 8.9. High water pH increases the rate of hydrolysis of many pesticides including mancothane (Al-Mughrabi *et al*., 1992; Al-Mughrabi and Qrunfleh, 2002). This means that the recommended dose of mancothane is lower due to alkaline hydrolysis before the fungicide is applied, but a possible consequence of this is that more isolates withstand the lower dose, becoming more resistant or less sensitive (Al-Mughrabi and Gray, 1995b). A programme for the control of early blight in Jordan should therefore include the recommendation to use mancothane at a higher concentration and to alternating it with another foliar fungicide (e.g., chlorothalonil, azoxystrobin, or TPTH).

Several approaches to controlling *A. solani* infection have been tested. Workman and Harrison (1980) found that delaying harvest to ensure tuber maturity and reduce harvest injury significantly reduced infection. Pre-harvest burning to destroy potato vine residue and to heat the soil, as well as the pre-harvest application of certain chemicals to the soil surface also significantly reduced tuber infection by *A. solani* (Lahman *et al.* 1981; Lahman *et al.,* 1982).

Integrated disease management practices are essential to prevent the onset or to assist in the control of early-blight. Growers can avoid early season infection by using blight-free seed and destroying potential sources of inoculum such as cull piles and volunteer plants. Tubers left in the field should be left to freeze and not disked or plowed under. Manure from culls fed to livestock should not be returned to the fields to serve for potato production. Planting resistant potato varieties and rotating with non-host crops may also help to eliminate the source of inoculum. The timing of fungicide applications (starting early, before the rows fill in), the frequency of application, and the application rate should conform to label recommendations. Alternating mancothane with fungicides having a different mode of action and a different chemistry may delay the build-up of strains resistant to mancothane. Top-killing of the vines at least two weeks before harvest allows the tubers to mature and reduces bruising while handling. A balanced fertilizer program should be the goal, one that will not delay maturity or increase susceptibility to early blight. Petiole analysis for nitrate content can be helpful in regulating fertilizer rates.

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