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Effect of acetochlor treatment on Fusarium wilt and sugar content in melon seedlings *

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Abstract

Pretreatment of soil with the herbicide acetochlor at 0.1–1 $\mu g\ g^{-1}$ significantly decreased incidence of wilt due to *Fusarium oxysporum* f. sp. *melonis* in melon seedlings. Glucose, fructose and sucrose increased in leaves of inoculated and uninoculated melon plants following acetochlor treatment. The increase in sugar levels in stems and roots was less pronounced. Light intensity affected sugar content and disease incidence. The percentage of diseased plants was significantly higher in untreated plants grown under 165 $\mu E\ m^{-2}\ sec^{-1}$ compared to plants grown under 300 $\mu E\ m^{-2}\ sec^{-1}$. Lowering light intensity resulted in reduction of levels of total sugars on the third and sixth day after inoculation. Acetochlor had little or no effect on growth rate or sporulation of the pathogen in culture. The colonization rate of diseased plant stems by the pathogen was similar in herbicide-treated and untreated plants, thus excluding the possibility that disease reduction by the herbicide is related to direct fungitoxicity.

Introduction

Herbicides may increase or decrease disease caused by soilborne pathogens [Katan and Eshel, 1973; Altman and Campbell, 1977]. Dinitroanaline herbicides, such as trifluralin and dinitramine, decrease wilt diseases caused by *Fusarium* and *Verticillium* pathogens [Grinstein *et al.*, 1976, 1984; Cohen *et al.*, 1986, 1987]. The incidence of bacterial wilt and of bacterial canker diseases in tomatoes was also reduced following treatment with dinitramine [Cohen *et al.*, 1992b]. In contrast, the dinitroaniline herbicides trifluralin and ethalfluralin increased the incidence of Rhizoctonia-caused damping-off in cotton [Neubauer and Avizohar-Hershenzon, 1973] and net blotch of peanut pods [Ben-Yephet *et al.*, 1991], respectively.

Physiological and anatomical changes in the herbicide-treated plants might be related to the induced increased resistance of the plants. The increase of tomato plant resistance to Fusarium wilt was partly explained by the accumulation of antifungal substances as a response of plants to herbicide treatment [Grinstein *et al.*, 1984]. Reduction in Fusarium wilt incidence in muskmelon plants treated with dinitramine was linked to suppression of ethylene production [Cohen *et al.*, 1986]. Reduced incidence of wilt diseases induced by a range of chemicals, including dinitroaniline herbicides, was accompanied by growth retardation [Buchenauer and Erwin, 1981; Cohen *et al.*, 1987]. These effects could be reversed by foliar application of gibberellic acid [Cohen *et al.*, 1987], suggesting a possible relationship between inhibition of gibberellic acid biosynthesis and resistance to wilt.

The chloroacetamide herbicides such as acetochlor are used in a variety of crops, mainly as pre-emergence or preplant, soil-incorporated herbicides [Fuerst, 1987]. These chemicals alter several physiological processes including gibberellic acid biosynthesis and sugar accumulation. Acetochlor induced resistance to Fusarium wilt in tomato, muskmelon and watermelon plants [Cohen *et al.*, 1992a] and was found to have

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effect on glutathione content in this plant-pathogen system [Bolter *et al.*, 1993]. Horsfall and Dimond [1957] suggested that some wilt diseases are 'low-sugar diseases' while others are 'high-sugar diseases', that is, susceptibility is favored by low or high sugar content in tissue, respectively. Fusarium wilt of tomato was classified as a low-sugar disease. Low light intensity or short photoperiod markedly decreased resistance, whereas root damage that causes sugar levels to rise in stems, increased resistance [Bell and Mace, 1981].

The purposes of this study were to evaluate the changes in sugar content in melon seedlings and to examine the relationship between sugar content in seedlings and their response to Fusarium inoculation as affected by treatment with the herbicide acetochlor.

Materials and methods

Herbicides and herbicide application. In this study, the chloroacetamide herbicide acetochlor was used. Water emulsions of the herbicide was mixed with sandy loam soil, tested to be free of soil-borne pathogens (0.6% organic matter; 3.8% clay; pH 7.9; field capacity 9%), to give the desired concentration of active ingredient as described by Cohen *et al.* [1986].

Plant inoculation and disease evaluation. Seeds of muskmelon (*Cucumis melo* L. cv. 'En Dor') were sown in herbicide-treated soil or in untreated control soil. One day after emergence, seedlings were removed and washed thoroughly to remove adhering soil and traces of the herbicide. Plants were transplanted into 250 cm^2 plastic pots, containing herbicide-free soil after being inoculated by dipping their roots for 2 min in a 10^6 ml^{-1} conidia suspension of *Fusarium oxysporum* Schlecht f. sp. *melonis* (Leach & Currence) Synd. & Han. [Cohen *et al.*, 1986]. In all experiments plants were grown in a growth chamber (5 pots with 8 seedlings per pot). Lighting was provided by high-pressure sodium vapor bulbs. Light intensity was 300 $\mu E\ m^{-2}\ sec^{-1}$ during a 12-h photoperiod, at a temperature regime of 25° in the day and 20 °C at night. Light intensity of 165 $\mu E\ m^{-2}\ sec^{-1}$, for the experiments conducted to test the effect of light on disease and sugar content, was provided by growing plants under the same conditions but under shading nets transmitting 55% of light incidence. Percentage of wilted seedlings was recorded periodically for 15 days.

Carbohydrate measurements. Three, five or six days after inoculation, in respect to the purpose of the individual experiment, one g fresh weight of melon plant organs (root, stem or leaves) were washed and extracted three times in hot 80% ethanol. After evaporation, reducing sugars and sucrose were measured by the methods of Miller [1959] and Van Handel [1968], respectively. Starch was assayed after amyloglucosidase treatment of the ethanol-insoluble fraction, using the method of Thivend *et al.* [1972]. Soluble sugars were also analyzed by HPLC [Schaffer *et al.*, 1991], in a system that consisted of a LDC/Milton Roy Constametric III pump and refractometer set to a detection limit of 2 μg. An Alltech 700 CH carbohydrate column heated to 90 °C, with double distilled H_2O delivered at 0.5 ml min^{-1}, was used. Eluted sugars were identified by their retention times and quantified according to standards. Data shown are average of 3 replicates.

Effect of acetochlor on Fusarium oxysporum *f. sp.* melonis *in culture and on its colonization of melon seedling tissue.* One ml of sterile aqueous solution of acetochlor at various concentrations was mixed with 9 ml molten potato dextrose agar (PDA) or yeast extract - glucose agar (YEGA) medium to give the desired final concentrations. A 5 mm diameter disc of a plate culture of *F. oxysporum* f. sp. *melonis* was placed in the centre of Petri dishes (10 replicates for each concentration). After 5 days incubation at 27 °C, percentage inhibition of fungus growth was calculated by comparing radial growth with that grown on medium without acetochlor. Conidia were harvested from fungus grown on herbicide-containing or herbicide-free media. The conidia yield from both treatments were assessed using a hemocytometer, and conidial suspension was used to inoculate melon seedlings.

Five days after inoculation, seedlings were harvested and stems were divided into lower and upper segments. After external disinfection for 1 min in sodium hypochlorite (10g l^{-1} available chlorine) followed by two rinses in sterile water, each segment was cut into five sub-segments and placed on a pentachloronitrobenzene medium selective for Fusarium [Alon *et al.*, 1974]. After 5 days incubation at 27 °C, the percentage of segments yielding the pathogen was determined.

Statistical analysis. All experiments were repeated at least twice. Since the trend was similar, the results of only one experiment were presented. Significance (*P*

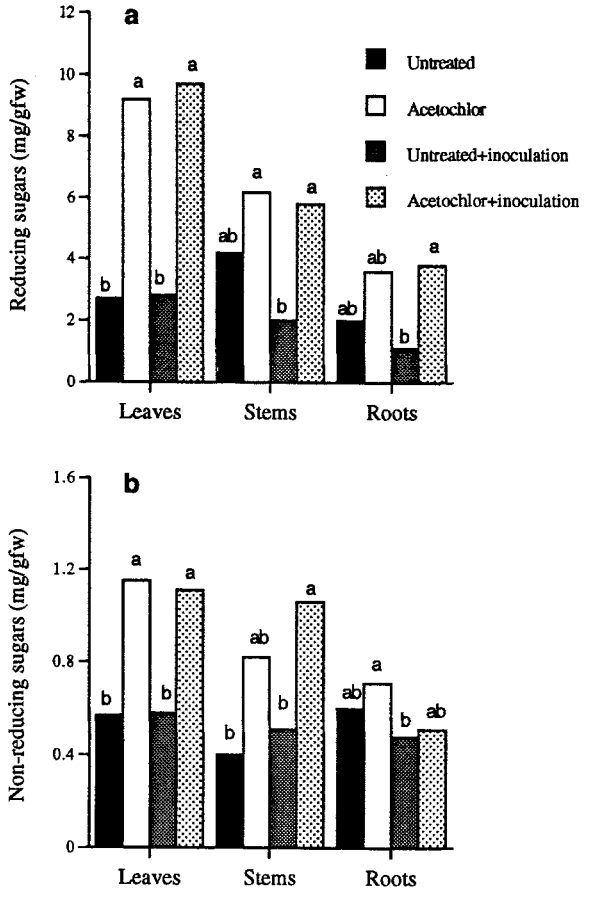

Fig. 1. Levels of reducing sugars (a) and non-reducing sugars (b) in muskmelon plant organs (leaves, stems and roots) as affected by acetochlor treatment at 1 μg g^{-1} soil and/or inoculation with *Fusarium oxysporum* f. sp. *melonis*, 5 days following inoculation. For each plant organ, columns with a common letter do not differ significantly (P = 0.05). fw = fresh weight.

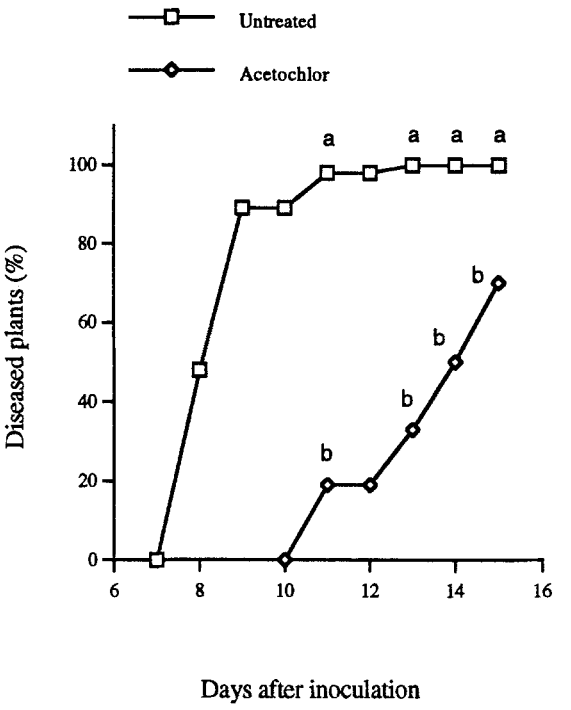

Fig. 2. Effect of acetochlor at 1 μg g^{-1} soil on disease incidence in muskmelon plants following inoculation with *Fusarium oxysporum* f. sp. *melonis*. At each date, values marked with the same letter do not differ significantly (P = 0.05).

= 0.05) of differences in sugar content and seedlings mortality were determined using Duncan's multiple range test.

Results

The effect of acetochlor and inoculation with F. oxysporum *f. sp.* melonis *on levels of sugars and starch in muskmelon plants.* Acetochlor (1 μg g^{-1} soil) significantly increased levels of reducing sugars in the treated plant organs (Fig. 1a). Inoculation with *F. oxysporum* f. sp. *melonis* had no significant effect on sugar levels. Similarly, acetochlor significantly increased levels of nonreducing sugars in in leaves and stems of the treated plants while inoculation had no effect (Fig. 1b). Pretreatment with acetochlor resulted in a delay in disease progress and a significant decrease in disease incidence during 15 days after inoculation (Fig. 2). In other experiments, acetochlor at 0.1 μg g^{-1} reduced disease incidence similarly to treatment at 1 μg g^{-1}, thus confirming earlier results [Cohen *et al.*, 1992a].

Acetochlor affected sugar composition in muskmelon plants. Glucose, fructose and sucrose levels in leaves of inoculated and noninoculated plants were increased to different levels by the herbicide treatment (Fig. 3). Acetochlor also significantly increased the level of fructose in stems of inoculated plants. However, it did not alter sugar levels in roots, regardless of treatment. Inoculation had no effect on the levels of these sugars.

Starch levels were in the range of 13–19 mg g^{-1} and were not affected by acetochlor treatments or by inoculation.

Effect of light intensity on Fusarium wilt incidence and sugar content in muskmelon seedlings. Disease incidence was affected by light conditions in the growth

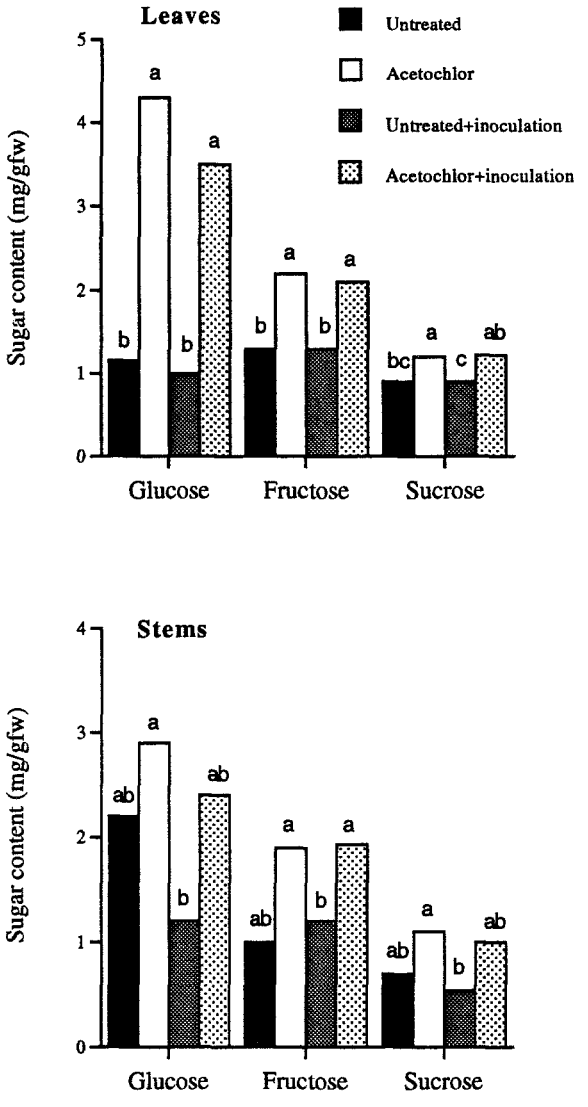

Fig. 3. Levels of glucose, fructose and sucrose in the leaves and the stems of muskmelon plants, treated or untreated with acetochlor at 1 μg g^{-1} soil, 5 days following inoculation with *Fusarium oxysporum* f. sp. *melonis*. For each sugar and plant organ, columns with a common letter do not differ significantly (P = 0.05). fw = fresh weight.

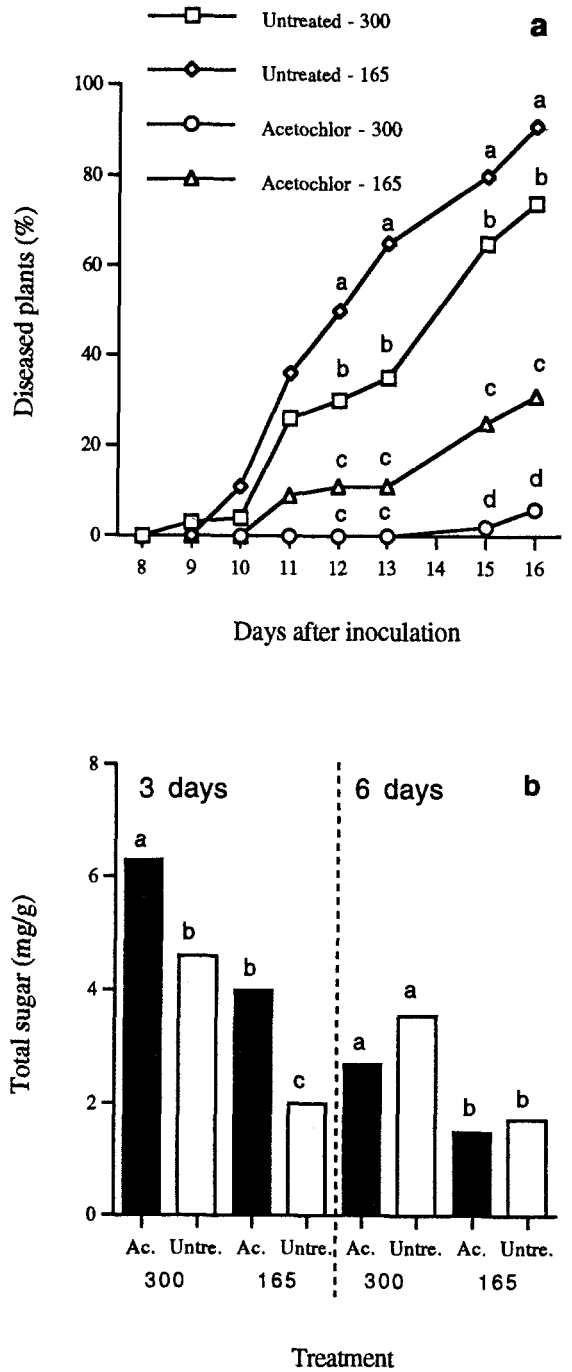

Fig. 4. Effect of light intensity and acetochlor treatment at 1 μg g^{-1} soil on Fusarium wilt incidence (a), and content of total (reducing and non-reducing) sugars (b) in muskmelon stems. In each date, values marked with the same letter do not differ significantly (P = 0.05). Ac. = Acetochlor; Untre. = Untreated and inoculated control; 165 and 300 are light intensities in μE m^{-2} sec^{-1} units.

chamber (Fig. 4a). In nontreated plants grown under 300 μE m^{-2} sec^{-1}, the percent of diseased plants was significantly higher than that of plants grown under 165 μE m^{-2} sec^{-1}. Light intensity had also significant effect on incidence of disease in herbicide-treated plants. At the end of the experiment, 16 days after the inoculation, only 5% of the seedlings grown under the high lighting were wilted, while disease incidence in the low light intensity was 31% (Fig. 4a). Reduc-

ing light intensity resulted in a significant reduction in the level of total sugars in stems on the third and sixth day after inoculation of untreated and herbicide-treated plants (Fig. 4b).

Effect of acetochlor on F. oxysporum *f. sp.* melonis *in culture and on pathogenicity.* Growth of the pathogen on YEGA or PDA amended with acetochlor at 0.1–1 $\mu g\ ml^{-1}$ was reduced by 9–13%. Sporulation of the pathogen was not significantly affected by the herbicide at these concentrations. Disease incidence in plants inoculated with conidia harvested from YEGA amended with acetochlor at 0.1 and 1 $\mu g\ ml^{-1}$ did not differ significantly from that caused by inoculation with conidia from unamended YEGA. Acetochlor treatment at 0.1 and 1 $\mu g\ g^{-1}$ soil had no significant effect on colonization of diseased plants by the pathogen: the percentages of segments from the non-treated and herbicide-treated (0.1 and 1 $\mu g\ g^{-1}$) plants yielding the pathogen were, respectively, 80, 92 and 70% from the lower stem and 55, 60 and 42% from the upper stem.

Discussion

Reduction of Fusarium wilt following exposure of melon seedlings to acetochlor is not related to a fungitoxic effect of the chemical. Acetochlor had no significant effect on the growth or sporulation and on pathogenicity of conidia of *F. oxysporum* f. sp. *melonis* grown in acetochlor-amended culture, as also found for dinitroaniline herbicides [Cohen *et al.*, 1992a]. Stems of muskmelon plants treated with acetochlor were colonized by the pathogen at the same rate as plants not treated with the herbicide. An absence of wilt symptoms in spite of plant colonization by the pathogen was also evident in earlier studies, in which induced resistance in muskmelon was obtained by pretreatment with dinitroaniline herbicides and growth retardants [Cohen *et al.*, 1987]. In a different system, Grinstein *et al.* [1984] found that trifluralin reduced wilt incidence in tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* and suppressed colonization of the plant tissues by the pathogen. In melons and tomatoes inoculated with Fusarium, genetically-resistant plants were colonized in a similar manner as herbicide-treated plants.

An increase in reducing sugars (glucose and fructose) and sucrose was evident in this study in acetochlor-treated melon seedlings. This may result from acetyl co-enzyme A inhibition, causing sugar accumulation, which has been shown to occur after treatment with the herbicide in a variety of plants [Fuerst, 1987].

Light intensity was used to manipulate sugar levels in plants. Disease incidence in plants grown under reduced light conditions (165 $\mu E\ m^{-2}\ sec^{-1}$) was significantly higher than in those grown under 300 $\mu E\ m^{-2}\ sec^{-1}$ (Fig. 4a). At low light intensity, the resistance phenomenon achieved by the herbicide was partially reversed. Disease increase was accompanied by lower levels of total sugars (Fig. 4b). Horsfall and Dimond [1957] suggested that Fusarium wilt of tomato is a low-sugar disease, that is, susceptibility is favored by low sugar content in the host.

Various mechanisms have been proposed for increased disease incidence with low sugar content of tissue. Patil and Dimond [1968] and Biehn and Dimond [1971] showed that sugar loading into tomato plants prior to inoculation, reduced disease incidence and repressed polygalacturonase synthesis by *F. oxysporum* f. sp. *lycopersici* in culture. It was suggested that reduced disease severity is a result of lowering the pathogen's ability to produce the enzyme. Similar results were reviewed also by Puhalla and Bell [1981]. Reduction in disease incidence due to herbicide treatment shown in the present study was closely connected with the accumulation of sugars. This was evident especially 3 days after the inoculation (Fig. 4b). This may suggest the involvement of sugars as part of the acetochlor-induced resistance phenomenon.

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