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# Effect of Ethylene Diurea on the Growth of Some Plant Pathogenic Fungi in Culture

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Ethylene diurea (EDU) is an antioxidant chemical, N - 2-(2 - oxo -1 -imidazolidinyl) ethyl - N - phenylurea, used for the protection of plants against ozone injury (CARNAHAN et al. 1978). A field trial of potato, Solanum tuberosum L., was conducted in Simcoe, Ontario during the summer of 1978 in which two varieties, Norchip and Kennebec, were exposed to ambient ozone and natural infection by early blight, Alternaria solani, and were sprayed with EDU. The EDU spray reduced early blight infection on the potato foliage. WUKASCH & HOFSTRA (1977) have shown EDU in agar failed to arrest growth of Botrytis species. EDU was reported (TEMPLE & BISESSAR 1979) to have no effect on the growth of Xanthomonas phaseoli colonies in plate cultures. Ozone causes leaf injury and reduces growth on potato (BRASHER et al. 1973; Heggestad 1973) and tomato (OSHIMA et al. 1977). Potato and tomato plants from which the fungus pathogens were obtained are likely candidates for antioxidant protection by EDU. This study was initiated to investigate the influence of EDU on Alternaria species of potato in plate and shake cultures and on other plant pathogenic fungi of tomato on different media.

## MATERIALS AND METHODS

EDU (50% WP) was supplied by E. L. Jenner (E.I. Du Pont de Nemours and Co., Wilmington, DE 19898). A. solani (Ell. and G. Mart), Jones and Grout, a potato isolate, was provided by R. J. Lukens, Chevron Chemical Co., Richmond, CA., and Alternaria alternata Fr. Keissl was obtained from the Canadian National Collection of fungus cultures, Ottawa. A. solani was isolated from natural infected potato leaves in Stouffville, Ontario. Botrytis cinerea, Septoria lycopersici and Stemphylium botryosum are some major pathogens recovered from tomato plants in southern Ontario. Isolates were maintained on potato dextrose agar (PDA) and stored at 5°C. Fungi were grown on PDA, malt extract agar, V-8 juice agar, or malt extract broth containing varying concentrations of EDU from 0-500 ppm. To each medium 0.1% surfactant, Tween - 20 (ICI Inc., Wilmington, DE 19897) was added. The pH was adjusted to 7.2 with 1% NaOH solution before autoclaving at 15p.s.i. for 20 min. Plates or flasks were inoculated with 4-mm discs taken from the edge of actively growing 4-day-old agar plate cultures.

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Cultures were incubated at ambient temperature (25 + 1C$^0$); light regime was either continuous fluorescent light or 14h light followed by 10h darkness for 7-14 days. The growth rates were determined by measuring diameter expansion or mass dry weight from liquid shake (Burrell wrist action) cultures of the fungi. Seven replicates repeated five times were made of all treatments in agar plate cultures.

Statistical analysis of variance and Duncan's Multiple Range test were used to test the significance of growth response among the treatments for each isolate.

## RESULTS AND DISCUSSION

Rate of growth in mm per 100 h (4 day) or mass dry weight in milligrams were calculated for each species grown at 0, 250 and 500 ppm active EDU concentration. The response of each fungus tested in vitro is shown in Table 1. A 1% level of significance was obtained among the treatments. There was an interaction between EDU and the plant pathogens. A significant decrease in total mass weight was also observed in liquid shake cultures with all isolates. Alternaria solani from naturally infected potato at Stouffville was the most sensitive to EDU. The fungus is not killed in the substrate containing EDU but it is prevented from growing as long as it remains in contact with the antioxidant compound. It will grow or germinate however, when removed from contact with EDU. Inoculum from cultures containing 1000 ppm EDU active was found to be viable when transferred to PDA plates.

TABLE 1. The effect of different concentrations of EDU with Tween-20 on the colony diameter (mm)$_A$ growth of the plant pathogens on PDA plates for four days[A].

| | EDU Concentration (ppm, active) | | |
|---|---|---|---|
| Isolate | 0 | 250 | 500 |
| POTATO | | | |
| Alternaria solani[C] | 17.0 | 8.0 | 5.0*[B] |
| Alternaria solani[D] | 20.2 | 17.1 | 12.2* |
| Alternaria alternata[E] | 22.6 | 16.1 | 14.7* |
| TOMATO | | | |
| Botrytis cinerea | 70.0 | 36.1 | 22.4* |
| Septoria lycopersici | 31.3 | 23.4 | 20.0* |
| Stemphylium botryosum | 30.1 | 21.1 | 15.6* |

A Values are the means from seven replicates repeated five times.

B Asterisk (*) indicates that values are significantly different, P = 0.01 according to Duncan's Multiple Range test.

C Isolate from naturally infected potato at Stouffville, Ontario, 1979.

D Isolate from R. J. Lukens, Richmond, CA.

E Isolate obtained from Canadian National Collection, Ottawa, Ontario.

All test species of Alternaria, Botrytis, Septoria and Stemphylum growth on 1 g/L of EDU in PDA were pathogenic when inoculated to Norchip potato or Ottawa 78 tomato plants in a clean-air-greenhouse. A reasonable explanation for Botrytis cinerea on EDU growth reduction in this study could be attributed to the pathotype sensitivity to the chemical. In this investigation the concentration of 500 ppm active which arrested the growth of the fungi tested in vitro was the dose used in field plot experiments to prevent ozone injury on bean (CARNAHAN et al. 1978) potato (CLARKE et al. 1978) and onion (WUKASCH & HOFSTRA 1977).

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