IN VITRO AND IN VIVO EVALUATION OF FUNGICIDES FOR CONTROL OF STEMPHYLIUM BOTRYOSUM F. LACTUCAE ON LETTUCE LEAVES

By

J. SIVAN* and RIVKA BARKAI-GOLAN**

Maneb was the most efficient chemical in inhibiting germination of Stemphylium botryosum f. lactucae spores in vitro. At 0.1 ppm it inhibited germination of all spores in the original population. Growth on PDA was markedly inhibited by 100 ppm daconil or maneb, with ca. 90-95% spore mortality. The inhibitory effects of chemicals at sublethal doses on the rate of colony growth resulted both in retarded radial growth and in prolonged incubation period in culture.

Maneb at 100 ppm and daconil at 10^4 ppm had an inhibitory effect on inoculated leaf discs only when applied immediately after inoculation, whereas sodium dimethyldithiocarbamate (SDMC) inhibited fungal development at 10^4 ppm both immediately after inoculation and 24 h later. SDMC kept the tissue very fresh during the experimental period.

Fungal development in inoculated lettuce heads was prevented by 10^3 ppm maneb or SDMC when applied before inoculation, but not when applied 24 h after inoculation, although in in vitro tests germinating spores were more sensitive to maneb than non-germinating ones.

KEY WORDS: Spore viability; sensitivity of germinating spores; fungistatic concentrations.

INTRODUCTION

S. botryosum f. lactucae Padhi & Snyder is one of the main fungal pathogens of lettuce, causing serious damage during prolonged storage (1). The fungus attacks the old external leaves of the lettuce head in the field. Subsequently, it penetrates to adjacent leaves, but the "heart" — which includes the youngest leaves — remains healthy (6). In the field the disease is characterized by small (2-3 mm) round spots, usually with concentric rings. However, during prolonged storage the spots lose their limited nature and tend to spread over most of the leaf, thus reducing possibilities for lettuce export.

The present work describes a study of the effect of some chemicals on Stemphylium development in vitro and in lettuce leaves.
MATERIALS AND METHODS

The following chemicals were tested for their effects on inhibiting fungal development:
Maneb: manganese ethylene - 1,2-bisdithiocarbamate;
Daconil: tetrachloroisophthalonitril;
SDMC: sodium dimethyldithiocarbamate;
Captan: N-trichloromethylmercapt-4-cyclohexene-1,2-dicarboximide;
Zineb: zinc ethylene - 1,2-bisdithiocarbamate;

Spores of S. botryosum f. lactucae were collected for both in vitro and in vivo studies, from a 14-day single-spore culture maintained at 24°C on potato dextrose agar (PDA).

Suspensions containing 10³ spores/ml of distilled water were prepared by counting spores in a Howard mould-counting chamber and confirming the number of viable spores by seeding suitable volumes on PDA, as described previously (2).

In vitro tests

Fungicides at concentrations of 0.2 to 2.10^4 ppm were mixed with the spore suspension, 1:1. The percent of spore germination under the effect of the various concentrations of fungicides was determined after 12 h at 24°C. Each treatment comprised nine replicates.

The effect of chemicals on the viability of fungal spores was tested by spreading aliquots of 0.25 or 0.5 ml of the fungicide-spore mixtures onto the surface of PDA in each of three petri dishes. The developing colonies were counted after 48 h at 24°C and again after 96 h, in order to locate colonies with retarded development. Viability of spores in the untreated controls was considered as 100%.

In the course of viability tests, the sensitivity to maneb of germinating spores as compared with non-germinating spores was tested. Spore suspensions in distilled water (10³ spores/ml) were held in a shaking bath at 25°C for 0, 2, 4, 6 and 8 h, after which their viability under the effect of 10 ppm maneb was tested as described above.

The effect of the fungicides at 10, 100 or 1,000 ppm on the colony rate of growth in vitro was tested on PDA. Inocula of uniform size (3-mm diameter) were taken from the periphery of 12-day-old colonies growing on PDA and including both mycelium and spores. Radial growth of the colonies was measured, in three replicate tests, during 8 days at 24°C. A control dish containing PDA without any fungicide was used in each test.

In vivo tests

Sixteen-mm-diameter discs, cut aseptically from external leaves of lettuce (Lactuca sativa L. var. Romaine) were placed in petri dishes on filter paper soaked with sterile water. Leaf-discs were inoculated by spraying with a spore suspension of 5.10⁴ spores/ml. Inoculations were followed by fungicidal spray treatments, either immediately after inoculation or 24 h later, at 100, 10² or 10⁴ ppm. Inoculated discs left otherwise untreated, or uninoculated discs, were used as controls. There were ten...