Survey and Screening of Benomyl-Resistant Strains of Cercospora beticola in Minnesota and North Dakota

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INTRODUCTION

The incidence and severity of *Cercospora* leaf spot of sugar beet (*Beta vulgaris* L.), caused by *Cercospora beticola* Sacc, has increased in Minnesota and North Dakota during the last 3 years (2). The occurrence of benomyl-resistant strains of *C. beticola* has been reported from Texas (7), Arizona (6), southern Minnesota (1,5) and Greece (4). Benomyl-resistant strains of *C. beticola* have been shown to be cross-resistant to the related fungicides thiophanate and thiabendazole (3). The purposes of this investigation were to determine the geographical distribution of benomyl-resistant strains of *C. beticola*, and to develop an accurate and efficient bioassay for benomyl resistance.

MATERIALS AND METHODS

Fungicide resistance screening. Sugar beet leaves were collected weekly from early August through late September 1984 from 50 fields located throughout the Minnesota and North Dakota sugar beet production areas. Sample size for each field was 10 - 55, with an average of 18 leaves (1 leaf/plant). Leaves were collected from sites 16 - 24 ha in size. Sugar beet leaves collected from survey fields were immediately placed in 3.8 l plastic bags and temporarily stored in portable ice chests, until placed in an incubator at 4°C.

To indentify benomyl-resistant strains of *C. beticola*,

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leaves were scanned with a dissecting microscope (40X), and one sporulating lesion was cut from each infected leaf. A drop (0.08 ml) of sterile distilled water containing 100µg/ml of streptomycin sulfate (Sigma Chem. Co., St. Louis, MO) was placed on each excised lesion. A 25 µl glass capillary tube (Clay-Adams, Parsypany, NJ) was used to dislodge the conidia. The conidial suspension from each lesion was then spotted in a single row of 5 to 10 spots/row on 2% water agar plates (WA) (Difco, Detroit, MI) and on WA amended with either 5 µg/ml benomyl (methyl 1 butylcarbamyl-2 benzimidazole carbamate, Benlate 50-WP, E.I. DuPont de Nemours and Co., Wilmington, DE) or 10 µg/ml triphenyltin hydroxide (DuTer 47.5-WP, Thompson-Hayward Chemical Co., Kansas City, KA). Both media also were amended with streptomycin sulfate and penicillin G (Pfizer Lab. Div., NY, NY) each at 200 µg/ml to control bacterial contamination.

Conidial suspensions from 3-7 lesions were spotted in a single petri dish. The inoculated plates were incubated at 24 C in the dark for 24 hr and then observed for conidial germination. A plus (+) sign was assigned to conidia that germinated normally and a minus (-) sign to those which had distorted germtubes or failed to germinate. Germinating conidia were then transferred to potato dextrose agar (PDA; Difco) and stored at 4 C. At least 100 conidia were observed from each infected plant assayed.

Cross-resistant to thiabendazole and thiophanate-methyl by benomyl-resistant strains of *C. beticola*. Sterile distilled water suspensions of thiabendazole (2-4-Thiazolyl benzimidazole, Mertect 340F, MSD AGVET, Rahway, NY), benomyl (Benlate 50 WP), or thiophenate-methyl (Dimethyl 4,4-O-phenylenebis 3-thioalbophanite, Topsin M-50 WP, Pennwalt Chemical Corp., Fresno, CA) were mixed with sterilized PDA to obtain the 5 µg/ml desired fungicide concentration. Nine single conidium isolates resistant to 5 g/ml a.i. benomyl were obtained from the fungicide resistance screening. All isolates were cultured on PDA for 7-10 days at 25°C under constant fluorescent light. My-

celial circles (5 mm diam.) from the edges of the cultures were placed onto PDA or PDA amended with 100 µg/ml thiabendazole, benomyl, or thiophanate methyl. Each treatment was replicated at least six times, and colony growth was measured after 15 days.

RESULTS

Fungicide resistance screening. Sugarbeet isolates resistant to at least 5 ug/ml of benomyl were recovered from 37 of 50 fields located in 16 counties throughout the sugarbeet production areas of Minnesota and North Dakota (Figure 1). Benomyl-resistant strains from the 37 fields

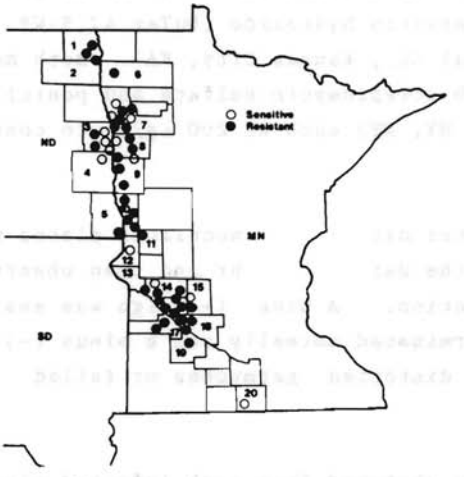

Figure 1. Distribution of Benomyl-resistant strains of *Cercospora beticola* in Minnesota and North Dakota during 1982. Counties surveyed:North Dakota (1-Pembina, 2-Walsh, 3-Traill, 4-Cass and 5-Richland). Minnesota (6-Marschall, 7-Polk, 8-Norman, 9-Clay, 10-Wilkin, 11-Grant, 12-Traverse, 13-Big Stone, 14-Swift, 15-Kandiyohi, 16-Chippewa, 17-Yellow Medicine, 18-Renville, 19-Redwood and 20-Fairbault).

surveyed in Minnesota were more numerous in the "Southern Region" (Minn-Dak and Renville factory districts) than in the "Northern Region" (Fargo-Moorhead, Hillsboro, Crookston, and Drayton factory districts) (Figure 2). There were 13 of 24 fields in the "Southern Region" with 50-99% resistant isolates compared with 2 of 26 fields in the

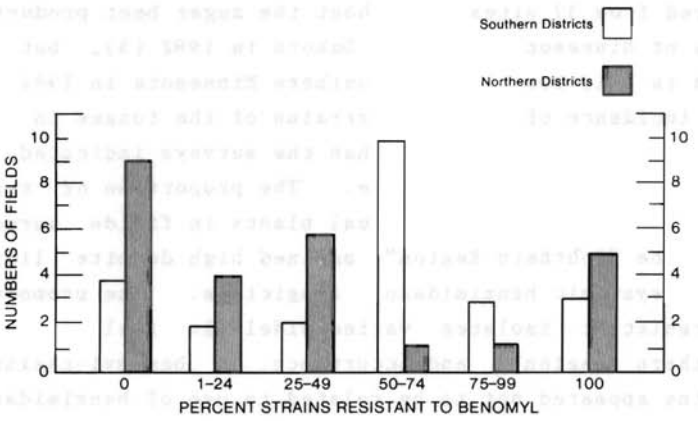

Figure 2. Survey of sugar beet fields in Minnesota and North Dakota containing isolates of benomyl-resistant strains of *Cercospora beticola* in 1982.

"Southern District" - Minn-Dak and Renville factory districts.

"Northern District" - Fargo-Moorhead, Hillsboro, Crookston and Drayton factory districts.

"Northern Region". There were 8 of 24 fields in the "Southern Region" with 0-49% resistant isolates compared with 19 of 26 fields in the "Northern Region" (Figure 2). One exception was the 3 of 24 fields in the "Southern Region" with 100% resistant isolates compared with 5 of 26 in the "Northern Region". None of the isolates tested was resistant to triphenyltin hydroxide at 10 ug/ml a.i. in vitro.

Cross-resistance to thiabendazole and thiophanate-methyl by benomyl-resistant strains of *C. beticola* . Colony growth varied among isolates and among fungicides. Colony diameter on unamended PDA varied from 55 to 66.3 mm for the nine isolates. Colony growth on PDA amended with 100 µg/ml benomyl, thiophanate-methyl, or thiabendazole was 65.9-95.0%, 83.3-97.6% and 35.9-51.8% of the control, respectively.

DISCUSSION

Benomyl-resistant strains of *C. beticola* were re-

covered from 37 sites throughout the sugar beet production areas of Minnesota and North Dakota in 1982 (5), but were found in only two sites in southern Minnesota in 1981 (1). The incidence of resistant strains of the fungus in both years was probably higher than the surveys indicated due to limitations in sample size. The proportion of resistant strains from individual plants in fields surveyed from the "Southern Region" remained high despite limited use of systemic benzimidazole fungicides. The proportion of resistant isolates varied widely in fields in the "Northern Region", and occurrence of benomyl-resistant strains appeared not to be related to use of benzimidazole fungicides inn previous years. All nine benomyl-resistant *C. beticola* isolates tested were cross-resistant to thiabendazole and thiophanate methyl, although the isolates reacted somewhat differently to each fungicide. The benomyl-resistant strains throughout the sugar beet growing areas of Minnesota and North Dakota were as virulent as benomyl-sensitive isolated (Percich, unpublished). The continued sole use of benomyl, thiabendazole or thiophanate-methyl fungicides in these sugar beet growing areas of Minnesota and North Dakota where benomyl-resistant strains of *C. beticola* have been identified may present an unacceptable risk factor resulting in an increased potential for fungicide-failure to control Cercospora leaf spot.

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