Persistence of Benomyl-Tolerant Strains of *Cercospora beticola* in the Absence of Benomyl

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ABSTRACT

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In 1976 and 1977, 98–100% of *Cercospora beticola* isolates obtained from diseased sugarbeets near Willcox, AZ, growing in benomyl-,triphenyltin-treated, or nonsprayed fields grew in PDA containing 5 μg a.i. benomyl per milliliter. Benomyl-sensitive isolates from Colorado were inhibited completely by 0.1 μg benomyl per milliliter. In 1978, 100% of the isolates from a triphenyltin-sprayed field also were tolerant to 100 μg benomyl per milliliter. The level of tolerance declined considerably between 1976 and 1977. In 1976, all isolates from benomyl-sprayed and nonsprayed fields grew in PDA containing 1,000 μg benomyl per milliliter, whereas only 71% of the isolates from the triphenyltin-sprayed field grew at that concentration. In 1977, only 1, 1, and 0% of the isolates from benomyl-sprayed, nonsprayed, and triphenyltin-sprayed fields, respectively, grew in PDA with 1,000 μg benomyl per milliliter. All of the isolates from 1978 grew in PDA cultures containing benomyl at 10 μg/ml, but none grew in those containing 100 or 1,000 μg/ml. Most Arizona isolates of *C. beticola*, whether from sprayed or nonsprayed fields, were 100–1,000 times more tolerant to benomyl in vitro than were sensitive control isolates from Colorado over the 3-yr study. Thus, benomyl-tolerant strains of *C. beticola* showed a high degree of persistence in the absence of benomyl, even in fields where triphenyltin was used for leaf spot control.

Additional key words: Beta vulgaris.

The sudden failure of benomyl to control sugarbeet leaf spot in Arizona in 1974 due to the development of benomyl-tolerant strains of *Cercospora beticola* Sacc. (7) raised a question about the persistence of benomyl-tolerant strains among natural populations of the pathogen no longer exposed to benomyl. Specifically, we wanted to determine if a 3-yr moratorium on benzimidazole use would greatly reduce or eliminate tolerant strains so that benomyl again could be used for disease control.

Recently, Dovas et al (1) reported that the frequency of benomyl-tolerant strains of *C. beticola* in Greece did not decline over a 3-yr period in the absence of benomyl, even when triphenyltin was used for leaf spot control. In our study, we not only determined the frequency of occurrence of tolerant isolates, but also their level of tolerance as determined by bioassays in vitro.

MATERIALS AND METHODS

Three widely separated areas near Willcox, AZ were selected for our 3-yr study. In 1976 and 1977, in one area a moratorium was placed on the use of benzimidazole fungicides and only triphenyltin hydroxide (TPTH) was used for leaf spot control. In a second area, sprays with benomyl were continued. No fungicides were used in experimental fields of Spreckels Sugar Division of the Amstar Corporation, which comprised the third area of study. In 1978, sugarbeets in all three areas were sprayed with TPTH for leaf spot control.

Each year, sugarbeets planted in March developed leaf spot by midsummer, and fungicides were applied in the designated areas according to normal fungicide spray schedules. Generally, these consisted of one to two benomyl applications (21-day interval), or two to three applications (10–14-day intervals) of TPTH depending on the area. In September of 1976 and 1977, a field in each area was used to randomly collect 100 *Cercospora*-infected sugarbeet leaves. In 1978, only the TPTH-treated field was sampled.

Sporulation of the fungus was induced on leaf spots by incubating leaves in plastic bags with a small amount of water for 48–72 hr at 22 C. An aqueous spore suspension was prepared from each of the three field leaf samples by transferring the spores collected from 100 leaf spots to 10 ml of sterile water. Petri dishes containing acidified 2% water agar were flooded with 5-ml serial dilutions of each suspension; excess water was decanted after 12 hr, and the dishes were incubated at room temp for another 12 hr. Randomly sampled single, germinating conidia of *C. beticola* from each field collection were isolated with the aid of a stereomicroscope and transferred to potato-dextrose agar (PDA) slants. After 72 hr of incubation, 100 pure *C. beticola* isolates from each field collection were transferred to PDA containing 5 μg active ingredient (a.i.) benomyl per milliliter to determine the frequency of tolerant isolates. The 100 isolates then were transferred to petri dishes of PDA containing 0, 1, 10, 100, or 1,000 μg a.i. benomyl per milliliter to determine the level of benomyl tolerance. Benomyl-sensitive isolates of *C. beticola* obtained from commercial sugarbeet fields in Colorado in 1969 were included as controls in all tests. Benomyl never had been used in the areas where these isolates were collected. Tolerance tests were repeated once for confirmation.

RESULTS AND DISCUSSION

In 1976, 1977, and 1978, regardless of source area, 98–100% of the Arizona isolates grew in PDA containing 5 μg benomyl per milliliter, whereas benomyl-sensitive control isolates from Colorado and two Arizona isolates in 1977 were inhibited by 0.1 μg/ml. The bioassay on PDA containing 5 μg a.i. benomyl per milliliter has proved to be a rapid and reliable means of detecting benomyl tolerance among isolates of *C. beticola*. In this and previous studies (6,7,8), all tolerant strains have grown in PDA containing more than 10 μg benomyl per milliliter.

The level of tolerance among Arizona isolates decreased considerably between 1976 and 1977 (Table 1). In 1976, 100% of the isolates from the benomyl-sprayed field grew on PDA containing 1,000 μg benomyl per milliliter, but only 1% from 1977 grew at this concentration. Similarly, the tolerance level of isolates from the triphenyltin-treated and untreated fields also decreased appreciably. Isolates collected in 1978 from the triphenyltin-treated area did not grow in PDA containing 100 or 1,000 μg benomyl per milliliter; when growth of *C. beticola* did occur, it was limited to the inoculum piece.

TABLE 1. Level of benomyl tolerance in vitro of *Cercospora beticola* isolates collected in 1976–1978 from Arizona

Benomyl[a] conc. (μg a.i./ml)	Isolates which grew at indicated benomyl concentration[b] (%)						
	1976			1977			1978
	B	T	C	B	T	C	T
0	100	100	100	100	100	100	100
1	100	100	100	98	100	100	100
10	100	100	100	99	100	100	100
100	100	71	100	96	99	84	TR[c]
1,000	100	71	100	1	0	1	TR

[a] Benomyl concentration in potato-dextrose agar.

[b] Isolates obtained from infected sugarbeet leaves from sugarbeet fields, sprayed with B (= benomyl), T (= triphenyltin hydroxide), or C (= non-sprayed). Means of two tests.

[c] TR = trace; growth limited to inoculum piece.

Either the strains having the highest level of benomyl tolerance have reduced fitness and do not survive in natural populations of *C. beticola* where benomyl has not been used, or they represent only a minute proportion of all strains and, thus, the chance of isolating them would be extremely small except where they have been selected by prolonged use of benomyl. Nevertheless, their apparent decrease over time may be academic, because isolates that tolerate 10 μg benomyl per milliliter in vitro can induce severe leaf spot in sugarbeet sprayed with normal field rates of benomyl (E. G. Ruppel, *unpublished*). Most Arizona isolates were 100–1,000 times more tolerant in vitro than were benomyl-sensitive control isolates from Colorado. Those Arizona isolates that proved to be benomyl-sensitive in 1977 were as sensitive as the control isolates.

The majority of benomyl-tolerant isolates in the nonsprayed area probably originated as primary inoculum within the area from commercial sugarbeet crops previously sprayed with benomyl. Since fields among the various areas were separated by a minimum of 2.8 km, spread of tolerant strains from treated to nonsprayed fields via wind seems unlikely (4), even though possible (3,5).

Although there was an apparent decrease in the level of benomyl tolerance among isolates of *C. beticola* over a 3-yr period, the decrease is considered to be of no practical importance. Like Dovas et al (1), we concluded that strains tolerant of field rates of benomyl exhibit a high degree of persistence once they have become dominant among natural populations of the fungus.

Continued use of benzimidazole fungicides cannot be recommended for areas where tolerant strains have developed, even after an extended moratorium on their use for sugarbeet leaf spot control. Previously, we (7) recommended TPTH as a substitute fungicide to control *C. beticola;* however, the recent development of triphenyltin-tolerant strains in Greece (2) eventually may preclude the use of TPTH for leaf spot control. The effectiveness of fungicide mixtures or the alteration of different fungicides in sugarbeet has not been explored; however, combinations of benomyl and protective-type fungicides were not effective for controlling *Venturia inaequalis* in apple orchards where a benomyl-tolerant strain predominated (10). The use of sugarbeet cultivars with genetic resistance to *C. beticola* is recommended for localities in which leaf spot is endemic. Where control by genetic resistance alone is not adequate, protective-type copper fungicides may have to be used, although they have not been as effective as the benzimidazole or tin compounds (9).

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