Occurrence of Iprodione-Tolerant *Fusarium nivale* Under Field Conditions

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ABSTRACT

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Twenty-one of 24 isolates of *Fusarium nivale* obtained from two golf course greens where iprodione failed to control development of Fusarium patch were able to grow on potato-dextrose agar amended with iprodione at ≥10 μg a.i./ml. Iprodione had been applied six times on both greens during 1977–1978 and four times during 1978–1979. Only one of eight isolates of *F. nivale* obtained from diseased turf that had never been sprayed with iprodione was able to grow on potato-dextrose agar amended with iprodione at 10 μg a.i./ml, and its growth was limited. Iprodione-tolerant isolates were cross-tolerant to vinclozolin and procymidone. Growth of tolerant isolates was significantly less than of sensitive isolates on unamended potato-dextrose agar. Tolerant isolates were pathogenic on Penncross bentgrass, and an application of iprodione at the highest labeled rate was ineffective in controlling disease.

Additional key words: dicarboximide fungicides, resistance

Fusarium patch, caused by *Fusarium nivale* (Fr.) Ces., is the most important disease on turfgrass in the Pacific Northwest. Foliar applications of iprodione, registered during 1979, provided excellent control of this disease (6).

During September–October 1979, an application of iprodione failed to control Fusarium patch on two greens of a golf course near Seattle, WA. Both greens were used by Rhône-Poulenc, Inc., during the 1977–1978 and 1978–1979 disease seasons to develop efficacy data in support of registration of iprodione against Fusarium patch.

From 8 November 1977 to 21 February 1978, six applications of iprodione were made to each green at about 3-wk intervals. Disease control was excellent when the fungicide was applied at 28.4 g (1 oz) and 56.8 g (2 oz) a.i./92.9 m^2 (1,000 ft^2). Starting on 28 September 1978, three applications of iprodione were applied to both greens at 3-wk intervals. The level of disease control decreased after the third application, and an application of phenylmercuric acetate was made on 27 November 1978 to bring disease development under control. One additional application of iprodione was made on 18 December, followed by applications of phenylmercuric acetate and mancozeb on 12 January and 20 February 1979, respectively.

The fungicide use pattern on these greens during the 1979–1980 disease season before disease development consisted of applications of iprodione at 28.4 g (1 oz) a.i./92.9 m^2 on 24 August, mancozeb at 181.4 g (6.4 oz) a.i./92.9 m^2 on 4 September, and iprodione at 56.8 g (2 oz) a.i./92.9 m^2 on 20 September. Disease developed on 3–5% of the total area of these greens following the application of iprodione on 24 August and 20 September. Fusarium patch did not develop on the other 16 greens that had received applications of the same fungicides during 1979–1980.

Samples of diseased turf were collected from the two greens on 17 October 1979. This report characterizes the tolerance of *F. nivale* isolates from the greens to iprodione and other dicarboximide fungicides.

MATERIALS AND METHODS

Sensitivity of isolates to iprodione. Twenty-four isolates of *F. nivale* were obtained from diseased turf collected from both greens where iprodione failed to control disease development. Eight isolates of *F. nivale* were also obtained from diseased turf near Puyallup, WA, that had never been exposed to iprodione. Isolations were made by placing surface-sterilized, diseased tissue on potato-dextrose agar (PDA). Isolates of *F. nivale* were maintained on PDA slants.

To determine the sensitivity of each isolate to iprodione, we grew all isolates on PDA and on PDA amended with iprodione at 50 μg a.i./ml. Mycelial plugs (5 mm in diameter) of each isolate grown on PDA were transferred to the center of each test medium. Fungicide-amended PDA, regardless of the fungicide used, was prepared by adding the fungicide dissolved in acetone to partially cooled, autoclaved media. An acetone check was included in each experiment. The volume of acetone added was always 1% of the volume of media.

Eight randomly selected isolates from the two greens that were able to grow on PDA amended with 50 μg of iprodione per milliliter and the eight isolates from the turf near Puyallup were tested for their ability to grow on PDA and PDA amended with iprodione at 1, 10, 20, and 40 μg a.i./ml. There were three replicates per isolate per media. Plates were incubated at 20–22 C for 4 days.

Tolerance to other dicarboximide fungicides. Three randomly selected iprodione-tolerant *F. nivale* and three randomly selected iprodione-sensitive *F. nivale* isolates were tested for their ability to grow on PDA and on PDA amended with iprodione, vinclozolin, or procymidone at 100 μg a.i./ml. Each medium was prepared and incubated as described above.

Pathogenicity. The pathogenicity of the three iprodione-tolerant and three iprodione-sensitive isolates of *F. nivale* was also compared on greenhouse-grown Penncross bentgrass. Conidial suspensions were obtained for each isolate by flooding 14-day-old colonies grown on PDA with sterile water. The resulting suspensions were filtered through four layers of sterile cheesecloth and adjusted to a final concentration of 1×10^6 conidia per milliliter. Twenty-five milliliters of a conidial suspension were sprayed onto 21-day-old Penncross bentgrass grown in 10.2-cm^2 plastic pots. Each isolate was used to inoculate six pots of turf, three of which had been sprayed 24 hr earlier with iprodione at the equivalent rate of 56.8 g a.i. (2 oz)/92.9 m^2. Pots were placed in a dew chamber at 20 C for 48 hr. Pots were then placed on a greenhouse bench, and the amount of disease development was determined 20 days after inoculation.

RESULTS

Sensitivity of isolates to iprodione. Twenty-one of the 24 isolates obtained from the two greens where applications of iprodione failed to control disease development during September and October 1979 were able to grow on PDA amended with iprodione at 50 μg a.i./ml. None of the eight isolates of *F. nivale* obtained from diseased turf near

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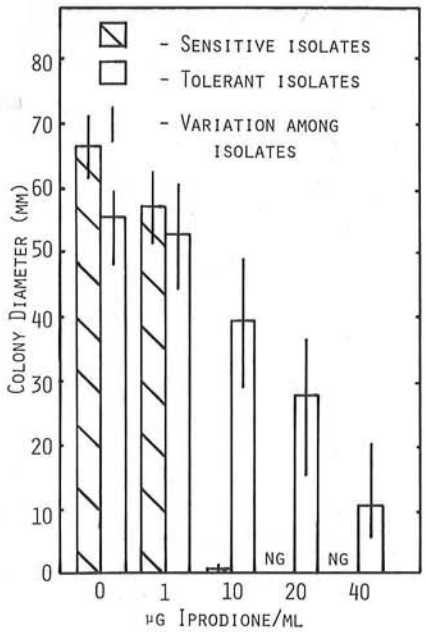

Fig. 1. Growth of eight iprodione-sensitive and eight iprodione-tolerant isolates of *Fusarium nivale* on iprodione-amended potato-dextrose agar after 4 days at 20–22 C. NG indicates no growth.

Table 1. Growth of three iprodione-sensitive and iprodione-tolerant isolates of *Fusarium nivale* on potato-dextrose agar amended with dicarboximide fungicides

	Colony diameter (mm) after 4 days[a]					
	Sensitive			Tolerant		
Fungicide (100 µg/ml)	1	2	3	1	2	3
None	75.8 x	71.5 x	71.5 x	61.0 y	59.8 y	63.0 y
Iprodione	0.0 y	0.0 y	0.0 y	20.3 x	27.5 x	25.0 x
Vinclozolin	0.7 z	0.3 z	4.3 z	22.0 y	47.0 x	38.3 xy
Procymidone	7.3 v	15.3 z	14.0 z	55.0 x	47.3 y	55.3 x

[a] Average of three measurements per isolate per media. Numbers in horizontal columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Pathogenicity of three iprodione-sensitive and three iprodione-tolerant isolates of *Fusarium nivale* on Penncross bentgrass

		Area diseased (%)[a]					
		Sensitive			Tolerant		
Treatment	Rate (g a.i./m²)	1	2	3	1	2	3
None	···	78.7 x	80.0 x	40.0 x	62.5 x	48.7 x	56.3 x
Iprodione	0.6	2.0 y	6.3 y	6.5 y	60.0 x	42.5 x	47.5 x

[a] Average of three pots per isolate per treatment. Pots of 21-day-old turf were inoculated with conidial suspensions containing 10^6 conidia per milliliter. Pots were incubated in a dew chamber at 20 C for 48 hr. Data were taken 20 days after inoculation. Numbers in vertical columns followed by the same letter are not significantly different ($P = 0.05$) as determined by analysis of variance.

Puyallup were able to grow on this medium.

Growth of the eight iprodione-tolerant isolates was significantly less than the sensitive isolates on PDA alone. As the concentration of iprodione in the medium increased, growth of the tolerant isolates decreased in proportion to concentration (Fig. 1). All eight of the isolates obtained from diseased turf near Puyallup were able to grow on PDA amended with iprodione at 1 µg a.i./ml; however, only one isolate was able to grow at 10 µg a.i./ml, and its growth was limited. The ED_{50} for reduction of growth on PDA by iprodione was 24 µg/ml for the iprodione-tolerant isolates and 2.9 µg/ml for the iprodione-sensitive isolates.

Tolerance to other dicarboximide fungicides. The three iprodione-sensitive isolates of *F. nivale* selected at random were unable to grow on PDA amended with iprodione and made limited growth on media amended with vinclozolin and procymidone. The iprodione-tolerant isolates were able to grow on PDA amended with iprodione, vinclozolin, or procymidone but had a slightly slower rate of growth compared with the iprodione-sensitive isolates on unamended PDA (Table 1). Additional testing has shown that those iprodione-tolerant isolates were able to make limited growth (11.3–19.3 mm) on PDA amended with iprodione at 1,000 µg a.i./ml after 4 days.

Pathogenicity. The three iprodione-tolerant isolates of *F. nivale* were pathogenic on Penncross bentgrass (Table 2). Only the tolerant isolates caused a significant amount of disease on the turf that had been sprayed with iprodione (Table 2).

DISCUSSION

Although tolerance to dicarboximide fungicides has been easily developed in vitro for several fungi, in vivo problems associated with tolerance after the use of these fungicides have not generally occurred (1–3,7,9,11,12). Several workers have shown that tolerant strains obtained in vitro vary in their fitness and virulence when compared with sensitive parent strains (8,10–12). In some cases, tolerance to this group of fungicides has resulted in a reduction or a loss of pathogenicity, indicating that tolerance may be a self-eliminating phenomenon under field conditions (2,4,7).

The failure of iprodione to control Fusarium patch on two greens that had applications of this fungicide during the preceding 2 yr indicates that tolerant isolates were able to survive from one disease season to the next. Current recommendations for preventing or delaying the development of tolerance to fungicides are based on the assumption that repeated applications of a single fungicide will result in the most rapid occurrence of tolerant strains (5). The development of iprodione-tolerant *F. nivale* strains reported here provides circumstantial evidence supporting this assumption.

Two other golf courses in the Seattle-Tacoma area also participated in the Rhône-Poulenc trials during 1977–1978 and 1978–1979. Disease control in both courses during 1977–1978 was excellent and was still good during the 1978–1979 season. On one of these courses during 1979–1980, applications of iprodione were ineffective in controlling Fusarium patch and were dropped from the fungicide program for this course. Isolates of *F. nivale* were not obtained from this course to confirm whether or not tolerance was responsible for the loss of iprodione effectiveness.

Applications of iprodione were made during 1979–1980 at the second course except from December to March, when other fungicides were applied. Iprodione continued to provide excellent control of Fusarium patch at this course.

Growth of the iprodione-sensitive isolates on PDA amended with vinclozolin and procymidone (Table 1) appears to be an example of acquired resistance. Hisada et al (8) noted a similar phenomenon for mycelium of *Botrytis cinerea* exposed to procymidone.

Increasing the concentration of iprodione in the medium beyond 40 µg/ml did not result in a significant change in the growth of the iprodione-tolerant isolates (Fig. 1 and Table 1). This may result because these concentrations exceed the solubility of iprodione (13 ml/L) in water.

The future effectiveness of iprodione against *F. nivale* on turf would appear to depend upon practices that would reduce the selection of iprodione-tolerant *F. nivale* from a sensitive population.

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