Fitness of *Venturia pirina* Isolates Resistant to Benzimidazole Fungicides

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ABSTRACT

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The competitive ability of carbendazim-resistant *Venturia pirina*, isolated from four pear orchards in Israel, was tested in greenhouse experiments and in the orchard, in the absence of the fungicide. Pear plants were inoculated with a mixture containing equal proportions of sensitive and resistant conidia. After 5–10 consecutive inoculations, the resistant isolates showed competitive ability equal to, or greater than, that of the sensitive wild type. *V. pirina* resistant to benzimidazole fungicides has persisted in pear orchards 4 yr after the withdrawal of these fungicides.

Additional key words: adaptability, benomyl, pear scab, tolerance.

Resistance of the pear scab pathogen, *Venturia pirina* Aderh., to benzimidazole fungicides was first found in four pear (*Pyrus communis* L.) orchards in Israel in 1975 (4). Since then, the *V. pirina* populations in many orchards have become resistant, forcing growers to switch to other fungicides.

Carbendazim (methyl 2-benzimidazole carbamate) resistance is attributed to a single gene mutation (5). All the mutants tested tolerated as much as 5,000 μM carbendazim in the growth medium; no intermediate level of resistance was detected even when the resistant mutant comprised only a small portion of the pathogen population. Thus, the history of fungicide application and the intensity of selection pressure imposed by it on the pathogen population, did not determine the level of resistance (in terms of tolerable fungicide concentration).

Now that benzimidazole-resistant populations of *V. pirina* exist in many Israeli pear orchards, it is important to determine how the populations might change in the absence of the fungicide. Greenhouse and field studies were undertaken to determine the competitiveness of resistant types in contrived and natural populations, and to estimate the probability that a mixed population might revert to the sensitive state upon withdrawal of the fungicide.

MATERIALS AND METHODS

The pathogen. The competitive ability of eight isolates of *Venturia pirina* was tested in four separate pairs, each consisting of a carbendazim-sensitive isolate and a carbendazim-resistant one (see Table 1). All the isolates, except S-33, originated from commercial pear orchards with a history of benzimidazole-fungicide treatments for scab control. The resistant isolates were similar to the sensitive isolates with respect to growth rate, sporulation, and morphology in vitro; they retained resistance in the absence of the fungicide, produced typical sporulating lesions on Spadona pear plants, and transmitted the carbendazim resistance to the new conidiospores (5). The following are details concerning the state of carbendazim resistance in the orchards from which the isolates were obtained: Pair 1—The two mono-ascosporic cultures (S-29 and R-9) were isolated from one orchard in the spring of 1976, when less than 10% of the *V. pirina* population was resistant to carbendazim; Pair 2—The sensitive monoconidial culture (S-26-1) was isolated in the spring of 1975, when most of the scab population in the orchard was sensitive, while the resistant one (R-6-12) was isolated from the same orchard 1 yr later, when almost all the population had become resistant due to continued benomyl sprays; Pair 3—These monoconidial cultures were isolated from two separate orchards in the spring of 1976; the source populations of the sensitive (S-1-2) and of the resistant (R-2-1) isolates rated, respectively, 80% and 100% resistant; Pair 4—Each isolate was obtained by collecting the bulk of the spores from several scab lesions on naturally diseased fruits. These isolates represented two *V. pirina* populations: one (that of S-33) had not previously been exposed to benzimidazole fungicides and remained sensitive, while the other (that of R-8) had been entirely resistant since 1974 (4).

Each isolate was propagated separately by inoculation on leaves of healthy potted Spadona pear plants by means of the filter paper disk technique (7). All the isolates produced sporulating lesions on

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the inoculated plants, indicating that they were pathogenic. Spores were collected from the lesions of each isolate and used for the preparation of primary inoculation mixtures for the competition experiments.

Determination of carbendazim resistance. Spores of carbendazim-sensitive *V. pirina* form short, distorted germ tubes on carbendazim-amended agar medium. In contrast, the spore germination and hyphal growth of resistant *V. pirina* is not impaired (5). Thus, sensitive and resistant spores were distinguished routinely by the shape of their germ tubes after incubation 1–2 days at 20 C on Difco potato-dextrose agar (PDA) supplemented with 5 μM of carbendazim.

Mixed inoculation and competition. The phenotype of each isolate was confirmed by plating spore samples from its lesions on PDA containing 5 μM carbendazim. Germination was always close to 100%.

The initial mixed inoculum of each series in the competition experiment, designated Inoculum No. 0, was prepared by combining spore suspensions containing equal numbers of spores of the two isolates constituting a pair. The intended (50%) proportion of each component in the initial mixed inoculum was confirmed by plating spore samples on carbendazim-amended PDA. Inoculum No. 0 of each pair of isolates was adjusted to ~25 $\times 10^4$ spores per milliliter and inoculated onto four potted pear plants (bearing a total of about 40 leaves) by use of the filter paper disk technique. The plants were kept in the greenhouse until scab lesions developed (7).

Spore samples were collected from individual lesions (about 20 lesions per inoculation) for phenotype determination on carbendazim medium. Then the spores of all the lesions of a pair were pooled, adjusted to about 25 $\times 10^4$ spores per milliliter, and inoculated onto a new batch of four healthy plants. This procedure was repeated five times with pairs 1, 2, and 3 (monosporic isolates) and ten times with pair 4.

Observations on *V. pirina* populations in commercial orchards. After carbendazim-resistant *V. pirina* had been detected in four pear orchards in two regions in Israel, the use of benzimidazole fungicides was discontinued in many pear orchards. Since 1976, these fungicides have been replaced by a mixture of triforine and captan in all the commerical orchards. During the years 1976–1979, scab-infected fruits were collected at harvest from commercial orchards, and the *V. pirina* populations were tested for carbendazim resistance by plating spores from individual lesions on a fungicide-amended medium.

RESULTS

Competition between carbendazim-sensitive and carbendazim-resistant isolates. The proportions of lesions yielding resistant spores in consecutive inoculations of pear plants by the different pairs of *V. pirina* isolates are depicted in Fig. 1.

The resistant mutants competed successfully against the sensitive wild types. In pair 2, the equal proportions of both competitors in the initial inoculum were maintained through the fifth generation of lesions. In the other two monosporic pairs, 1 and 3, the proportions of the resistant mutants increased gradually and reached 96–100% in the fifth generation of lesions; and the resistant isolate of pair 4 approached 100% in the tenth generation.

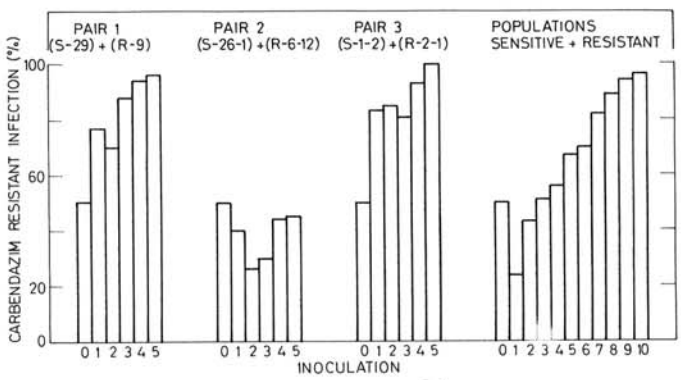

Fig. 1. Competition between carbendazim-resistant and sensitive isolates of *Venturia pirina*, on successive inoculations of potted pear plants.

Persistence of carbendazim-resistant *V. pirina* in commercial orchards in the absence of the fungicide. Since 1976, benzimidazole fungicides have been replaced by a mixture of triforine and captan in all the pear orchards in Israel. During the years 1976–1979, *V. pirina* populations in commercial orchards were monitored for carbendazim resistance. Results of 1977 showed that resistance had spread to about half of the surveyed orchards (31 of 59) (5). Table 2 summarizes the yearly changes in the percentage of carbendazim-resistant *V. pirina* in nine orchards. In Tel Zoffit, Har'el, and Daliya the initial level of 100% resistance remained 4 yr after the fungicide withdrawal, although sensitive *V. pirina* was present in nearby orchards. In orchards having initially mixed populations (Idmit, Rosh Pinna, Zar'it, and Yir'on) the proportions of resistant *V. pirina* fluctuated but were not selectively decreased. In the initially sensitive population at 'Ein HaShofet, located close to Daliya orchard with the resistant scab pathogen, carbendazim resistance was first detected 2 yr after the fungicide withdrawal. Similarly, small percentages of the resistant pathogen were first detected in 1977 in the orchards of Bar'am, Metulla, Lavee, Yif'at, Kefar haChoresh, and Hawwat Matityahu.

DISCUSSION

Four carbendazim-resistant isolates of *V. pirina* competed effectively against sensitive ones, in the absence of the fungicide, in artificially mixed inoculations of pear plants in the greenhouse. Three of the resistant isolates succeeded in taking over the sensitive pathogen populations. This indicates that carbendazim-resistant strains of a pathogen were not necessarily weaker (less fit) than the sensitive wild type.

TABLE 1. Pairs of isolates of *Venturia pirina* tested in competition experiments

Pair	Isolate[a]	Isolation	
		Year	Orchard
1	S-29	1976	Zar'it
	R-9	1976	
2	S-26-1	1975	Rosh Pinna
	R-6-12	1976	
3	S-1-2	1976	Idmit
	R-2-1	1976	Dovev
4	S-33	1976	Hawwat Matitiyahu
	R-8	1976	Tel Zoffit

[a] S, carbendazim-sensitive; R, carbendazim-resistant.

TABLE 2. Persistence of carbendazim-resistant *Venturia pirina* in commercial orchards in the absence of benzimidazole fungicides[a]

Orchard[b]	Resistant *V. pirina*[c] (%)				
	1975	1976	1977	1978	1979
Dovev	<1	100	100	NT[d]	NT
Idmit	<1	82	31	63	NT
Rosh Pinna	<1	100	86	NT	NT
Tel Zoffit	100	100	100	100	100
Zar'it	0	6	13	NT	NT
Har'el	100	100	100	100	NT
Daliya	–[e]	100	100	100	100
'Ein haShofet	–	0	20	38	NT
Yir'on	0	24	75	NT	NT

[a] All the orchards were sprayed with benzimidazole fungicides during 1975, but not in subsequent years.
[b] The first five orchards served as sources of *V. pirina* isolates used in the competition experiments (see Table 1).
[c] About 100 lesions per sample.
[d] NT, not tested, or orchard uprooted.
[e] –, no scab infections.

The competition experiments were aimed at prediction of population trends. Wolfe (9) questions the significance of such experiments under artificial conditions and argues that environmental variables are excluded that may affect the adaptability of the competing strains. However desirable, comparable field experiments cannot be performed with a perennial crop like the pear. Furthermore, a field experiment as such does not assure proper representation by a selected pair of isolates of the pathogen's populations (1), and it is also apt to be "contaminated" by external populations. The artificial conditions of our experiments facilitated more infection cycles than occur in nature per unit of time. The relevance of the results to actual situations can now be assessed by the parallel observations of orchard populations. During 4 yr, the resistant phenotype has proven to be well fitted to field conditions in the absence of the fungicide. In no case did it disappear or selectively diminish, but rather it showed good persistence in entirely or partially resistant populations. Its invasion into sensitive populations, and its establishment there in the absence of fungicide selection pressure, conformed with the results of the competition experiments, indicating that it might be more competitive than the sensitive wild type.

Reports on carbendazim-resistant strains of several plant pathogens indicate that in some cases their pathogenicity, competitiveness, and persistence in the absence of the fungicide are comparable or superior to those of the sensitive wild types (1,2,3). In another case, the tolerant strain declined after fungicide withdrawal, but resurgence to a high level was observed upon its reapplication (8).

After the resistant *V. pirina* had been detected in the orchard, the incorporation of conventional fungicides into benomyl-spray programs (in tank mixtures or in alternating sprays) did not prevent its increase within the scab population (6). Thus, in order to cope with the fungicide-resistance problem, the effort must be directed at the prevention or diminution of the initial build-up of resistant phenotypes.

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