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**Development and Epidemiology of
Fungicide-Resistant Plant Pathogens
in an Integrated Pest Management
Program for Pome and Stone Fruit
Production**

E. Shabi, J.M. Ogawa, J.D. Gilpatrick

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1. FOREWORD

The proposal for this cooperative research was presented at the end of 1978. At that time the main threat to agriculture from fungicide-resistant plant pathogens came from benzimidazole fungicides. During the last five years, fungicide resistance has increasingly become a problem and since 1979 plant pathogens have been developing resistance to newer, highly effective fungicides from the dicarboximide the acylalanines, and most recently from the ergosterol biosynthesis inhibiting (EBI) fungicides. This prompted efforts on the part of many research workers to acquire more knowledge on plant pathogen resistance to fungicides and to search for ways of coping with this threat to agriculture. In our research we have mainly investigated resistance to benzimidazole fungicides and especially to benomyl, the leading compound of this group. Thus, in this report we mainly use the term 'benomyl resistance' but our results and conclusions should be referred to the benzimidazole fungicides in general. The benomyl resistance of Monilinia fructicola in California caused some crop losses already in 1978. The threat of heavy losses to growers of stone fruit motivated the research in California, where about a quarter million acres have received benomyl applications since 1972. The efforts of Dr. Ogawa and his research group were directed at investigating Monilinia-resistance and providing economic control of the brown rot disease of stone fruit in California. Research in California is described by Dr. Ogawa, and includes seven manuscripts and four abstracts. The remaining chapters of this report are presented by Dr. Shabi, and include five manuscripts and eight abstracts.

Dr. J.D. Gilpatrick died in March 1982, while the project was in progress. The benefit of his experience and knowledge are sorely missed in the presentation of this final report.

2. ABSTRACT

Shabi, E., Ogawa, J.M., and Gilpatrick, J.D. 1983. Development and epidemiology of fungicide-resistant plant pathogens in an integrated pest management program for pome and stone fruit production. BARD Project I-26-79, Final Report.

Early detection of benomyl resistant Monilinia laxa in California and Venturia inaequalis in Israel before crop losses have occurred is reported. Methods of detecting fungicide-resistant isolates in the laboratory and in the orchard are described and discussed. The fitness of the resistant isolates is compared in vivo and in vitro to that of the sensitive populations. The in vivo research includes greenhouse experiments and data obtained from orchards. Genetic research in Venturia inaequalis was related to the presence of isolates from New York and from Israel with different levels of benomyl resistance. The importance of this genetic research to the nature of benomyl resistance and to its persistence is discussed. New fungicides were evaluated for scab and brown rot control and were found to be highly effective alternatives to benomyl.

3. INTRODUCTION

Apple and pear scab, brown rot, blossom blight and fruit rot were selected as model plant diseases for a study on the development and epidemiology of fungicide-resistant plant pathogens. The causal organisms are Venturia inaequalis, V. pirina, Monilinia fructicola and M. laxa. At the commencement of the project (September 1979), the state of resistance at the various localities were the following:

- 1) California: The brown rot of stone fruit caused by Monilinia spp. was treated mainly by benomyl. In a few peach orchards benomyl-resistant M. fructicola had been detected. These benomyl-resistant isolates were found to be insensitive to low levels of benomyl (>10 $\mu\text{g/ml}$ of benomyl in the poisoned media) and sensitive to higher benomyl concentrations in the in vitro tests. No resistance had been found up to that time in the population of M. laxa.
- 2) Israel: Although benomyl was the main fungicide used for the control of stone fruit blossom blight caused by M. laxa, no resistance had been detected in almond, apricot, plum or peach orchards. In apple, no resistance had been encountered in the population of V. inaequalis although benzimidazoles were used for apple scab control. From 1979 the benzimidazole fungicides were no longer recommended for the control of pear scab because benomyl-resistant V. pirina had been detected in most pear orchards in Israel.
- 3) New York: In cherry orchards in western New York, high level benomyl-resistant M. fructicola caused failures in brown rot control. The appearance of such resistance in cherry orchards four years after the introduction of benomyl for use against brown rot was attributed to the intensive use of benomyl by growers of stone fruit. At that time dicarboximide and EBT fungicides were found to be effective for brown rot control. In a few apple orchards benomyl-resistant V. inaequalis had been detected. Resistance to dodine in V. inaequalis excluded the use of this effective fungicide for apple scab control in New York.

4. RESEARCH RESULTS

I. CALIFORNIA (J.M. Ogawa, University of California, Davis)

Development and Epidemiology of Fungicide-Resistance in Monilinia fructicola and M. laxa on stone fruit.

ABSTRACT

Methods of detection, epidemiological studies, and ways of preventing or delaying the development of benomyl-resistant Monilinia spp. on stone fruit are reported. Detection research involved methods of identifying and characterizing for the first time benomyl-resistant M. laxa in California. Epidemiological studies related the presence of 1-4 ppm of benomyl-resistant M. fructicola isolates to their ability to compete effectively with benomyl-sensitive isolates in causing blossom blight and fruit rot of stone fruits. Benomyl-resistant lines showed less ability to compete with benomyl-sensitive lines. Prochloraz, a highly effective alternative to benomyl with a different mode of action, prevents development of benomyl-resistant Monilinia in dates and a slightly greater toxicity towards the benomyl-resistant isolates may help. Increases in population levels of benomyl-resistant Monilinia were not delayed by combination sprays of benomyl plus captan. Effective disease control with benomyl plus captan was possible in orchards with low population levels of 1 µg/ml of benomyl-resistant M. fructicola.

INTRODUCTION

The brown rot of stone fruits caused by Monilinia spp. is a serious disease that causes blossom blight, twig blight, green fruit rot, preharvest fruit rot and postharvest decay. During the administration period of the BARD grant (1979-83) severe brown rot epidemics occurred, especially in California peach and apricot orchards where the fungicide benomyl was used alone or in combination with captan. Surveys demonstrated that many isolates of M. fructicola had become resistant to benomyl. As part of this BARD project, methods were developed to detect,

characterize, and compare the benomyl-sensitive and benomyl-resistant isolates in epidemiological studies. Methods of control which have proved beneficial to California fruit growers are of special interest to growers in other countries such as Israel, where benomyl-resistance has not yet developed in Monilinia spp.

MATERIALS AND METHODS

Detection. The method of detection suggested by Ogawa et al. (27,28) was tested on a larger scale in California orchards where M. fructicola is resistant to benomyl at concentrations between 1 and 4 $\mu\text{g/ml}$. Benomyl is incorporated into Difco potato dextrose agar (PDA) plates. The results are expressed as minimum inhibition of mycelial growth within a four-day incubation period at room temperature ($24\pm 1^\circ\text{C}$). Isolates of M. fructicola were obtained from conidia on blossoms and fruit and from ascospores from apothecia collected under peach orchards in California. M. laxa isolates were from Israel and California. (For details see 4,9,16,23.)

Epidemiology. Epidemiological studies were carried out with conidia and ascospores from peaches and conidia from apricots. Field studies were conducted in a peach orchard located in San Joaquin County, and fruit used for experimentation were from nontreated controls in experimental plots located in San Joaquin, Fresno, and Yolo counties. Laboratory studies were conducted on the Davis Campus. (For details see 8,10,12,17).

Preventing or delaying development of resistance

Plots were located in isolated areas of commercial orchards in order to conduct experiments with randomized designs, including a nontreated control. Sprays were applied with a handgun to drip stage or with airblasting equipment. (For details see 1,11,13,14,17,23).

RESULTS

The results of the research are summarized here because manuscripts containing detailed descriptions of the results have been published, are in press, or have been prepared for submission. Copies are attached to this report. Highlights of the results include:

Detection: The method emphasizes the importance of collecting samples during or immediately after bloom and again before harvest. Isolates should be prepared immediately because saprophytic organisms predominate on samples held in storage for long periods. The test method described in FAO Method No. 26 involves the use of an easily available standard potato dextrose agar medium (PDA), such as Difco (27). To detect resistance, isolates are transferred to PDA amended with benomyl in concentrations from 1 to 4 $\mu\text{g/ml}$. Benomyl (proprietary) should be directly incorporated within the PDA before autoclaving (27,28). This method was used to detect benomyl-resistant M. laxa before its widespread occurrence (4,16). Populations of benomyl-resistant isolates within an orchard and the level of resistance may also be determined using this method.

Definite identification of the Monilinia species is essential for accurate detection of benomyl resistance. The formation of barrage (vegetative incompatibility) lines between unrelated isolates is useful for identification. The M. laxa isolates from Israel were found to be identical with typical isolates found in California and did not form barrages. However, Israeli M. laxa isolates formed barrages when placed next to M. fructicola (10).

Epidemiology. Fitness of the benomyl-resistant isolates was determined by studying the differential growth rates of benomyl-resistant and benomyl-sensitive Monilinia on synthetic medium (PDA) and on peach fruit (4,8). There was a greater difference between mean growth rates of benomyl-sensitive and benomyl-resistant isolates on peach fruit

than on PDA. There was no correlation between growth rates of isolates on PDA and on peach fruit. When benomyl-resistant and benomyl-sensitive isolates of M. fructicola were inoculated together on peach fruit, the resistant isolates were usually less fit (12). Isolates from orchards sprayed exclusively with nonbenzimidazole fungicides demonstrated that the proportion of benomyl-resistant lines had not diminished within a two-year period. The percentage of benomyl-resistant isolates within an orchard increased when benomyl was used exclusively or in combination with captan. There was no apparent delay in the development of resistance when captan was combined with benomyl, although when captan was used alone the population percentages remained unchanged. (1,11).

Control: Sprays with benomyl alone or in combination with captan did not effectively control brown rot in peach orchards when the benomyl-resistant isolates tolerated 1-4 $\mu\text{g/ml}$ benomyl and approximately 20% of the population was resistant (14). However, when benomyl-resistant isolates tolerated only 1 $\mu\text{g/ml}$ and 20% of the population was resistant, benomyl sprays controlled brown rot blossom blight, as did captan alone (11). Alternative fungicides with different modes of action from that of the benzimidazoles, such as prochloraz and captan, are needed in orchards with a high level of benomyl resistance and high population percentage resistant to benomyl. In cultural studies, both captan and prochloraz showed greater toxicity towards benomyl-resistant than towards benomyl-sensitive lines (1).

Laboratory studies testing the possible development of fungal resistance to ergosterol biosynthesis inhibitors and dicarboximides (13,14,24) indicate the need for establishment of baseline sensitivities before these chemicals are put into commercial use. Resistance to these fungicides has not been detected in commercial stone fruit orchards after two years of triforine registration and one year of iprodione registration.

Postharvest treatments using triforine have been highly effective in controlling decay caused by benomyl-resistant M. fructicola (15).

DISCUSSION

The laboratory method for detection of benomyl-resistant isolates of Monilinia resulted in early detection of benomyl-resistant M. laxa and has made possible the monitoring of both population percentages and levels of benomyl resistance in M. fructicola. However, the technique requires expertise and is very laborious. Other techniques which could be developed for rapid detection of benomyl resistance include enzyme-linked immunosorbent assays (ELISA) or direct morphological or biochemical tests. Without such rapid detection methods, adequate monitoring of large numbers of commercial orchards is not feasible.

Epidemiological studies in the laboratory using both in vitro and in vivo studies have demonstrated the reduced fitness of benomyl-resistant isolates of Monilinia. However, the widespread occurrence of the benomyl-resistant lines indicates the general fitness of these same benomyl-resistant lines in commercial orchards. In orchards where the use of alternative chemicals has removed the pressure for continued development of benomyl resistance, benomyl-resistant isolates have remained unchanged and exist in percentages between those of the resistant and the sensitive lines. Kato et al. of Sumitomo Chemical Company have recently reported a fungicide that can effectively control the benomyl-resistant isolates but not the benomyl-sensitive isolates of several fungi. If this compound shows similar effects on benomyl-resistant Monilinia, studies on the epidemiology of Monilinia could be greatly enhanced.

Studies on Monilinia control in blossoms, preharvest fruit rot and postharvest decay indicate the need for alternative nonbenzimidazole compounds such as triforine and iprodione, even though benomyl-resistance does not exceed concentrations of 1 to 4 µg/ml. Combination mixtures of captan with benomyl did not provide disease control nor delay the

increase in population percentage of benomyl-resistant lines. Captan is a far less effective fungicide than benomyl and did not provide the effectiveness necessary to reduce benomyl-resistant populations in the orchard. The need for benomyl in fungicide mixtures is questionable when highly effective materials such as triforine and iprodione are available. The cost of fungicide mixtures containing benomyl is difficult to justify when triforine and iprodione can effectively control both the resistant and the sensitive lines of Monilinia.

II. New York (J.D. Gilpatrick, Cornell University, NYAES, Geneva)

Resistance to EBI fungicides in Monolinia fructicola

The causal agent of brown rot in stone fruit, M. fructicola, has been responsible for heavy losses in Western New York State. Resistance to benomyl was reported from New York in 1977, before this BARD research proposal was submitted. The research in New York was directed towards the detection and induction of resistance to EBI fungicides, (for details see 13).

III. Israel (E. Shabi, Volcani Center, ARO, Bet Dagan)

Monitoring Monolinia laxa population for early detection of resistance to fungicides

Introduction

The causal agent of blossom and twig blight in stone fruit, M. laxa, is abundant on almond, apricot and plum. M. laxa has also been recorded from time to time in peach orchards. The chemical control of disease in Israel has been used mainly for apricots and plums, which have suffered heavy crop losses. Since 1975 the systemic fungicides benomyl and triforine have been used for M. laxa control. Field experiments have indicated that dicarboximide fungicides might be useful for the control of M. laxa. No benomyl-resistant isolates have been found in Israel.

The objectives of our research were:

- a. To monitor the population of M. laxa for benomyl resistance;
- b. To detect and induce M. laxa isolates resistant to dicarboximide fungicides (details in 2,14,24).

Materials and Methods

a. Benomyl resistance. Cankered branches of almond, apricot and plum were collected in autumn and winter from infected trees. M. laxa was isolated from the cankers and checked for benomyl resistance on 0.5 µg/ml benomyl. Test plots were set up in growers' orchards in order to study the effect of benomyl on the development of resistance. Three plots of almond, apricot and plum heavily infected with M. laxa were sprayed during four seasons with 250 µg/ml benomyl. The experimental plots were sprayed once per season, at early bloom stage. M. laxa sporodochia were transferred from infected blossoms to PDA amended with 0.5 µg/ml benomyl. In this way the M. laxa populations from the experimental plots were monitored for resistance. M. laxa from growers' orchards were also monitored for resistance by sampling infected blossoms in the spring (32).

Results

a. Benomyl resistance. During the four seasons of study, no resistant isolates were detected either in experimental plots or in growers' orchards. Although some apricot orchards suffered from heavy M. laxa infections despite benomyl and triforine spray applications, no resistant isolates were obtained.

Discussion

Benomyl has been used for M. laxa control since 1975 in apricot orchards and since 1979 in plum orchards. It seem that the single benomyl spraying in most orchards did not result in selection of a resistant mutant from M. laxa population. In some apricot orchards two fungicide applications were used for the control of blossom blight, one at early bloom stage and the other at full bloom. The recommended spraying regimen was to alternate benomyl with triforine or prochloraz, an EBI fungicide which was found to be very effective against M. laxa (32).

From the results of research on detection and induction of resistance to dicarboximide fungicides (2) it is clear that resistant isolates could be obtained easily in the laboratory. The fact that these resistant isolates from the laboratory are less fit than the wild type isolates indicates that alternation of fungicides may be a useful strategy for delaying or even avoiding resistance in the case of dicarboximide fungicides.

IV. Israel and New York (E. Shabi, Volcani Center and J.D. Gilpatrick,
NYAES, Geneva)

Development and epidemiology of fungicide-resistant Venturia spp.
in apple and pear

A. VENTURIA INAEQUALIS

INTRODUCTION

Benomyl-resistant V. inaequalis was found in a few orchards in Western New York State in 1978 and in Israel in 1981. Benomyl had never been the exclusive fungicide used for apple scab control in either of these countries. In both Israel and New York powdery mildew is an important disease of apple. Sulfur compounds, which are effective fungicides against powdery mildew and have some activity against scab, have been in use for many years in apple orchards. Summer apple diseases have been treated with captan, a fungicide which is effective for scab control. Dodine was used for scab control in New York; since 1968, however when apparent resistance of V. inaequalis to dodine was found in western New York, its use was restricted.

The objectives of our research were:

To monitor scab in apple orchards for early detection of benomyl resistance in V. inaequalis in Israel (for details see pp. 15-16).

To characterize the resistant isolates and their fitness (for details see 3,6,7,19,21,22 and pp. 17).

To study the genetics of benomyl-resistance (for details see 3,7).

To find effective replacements for dodine and benzimidazole fungicides for the control of apple scab (for details see 5,29,30,31).

To use the pathogenic specialization in V. inaequalis pathotypes for fungicide resistance research (for details see pp. 18, and Abstr. 18).

a. EARLY DETECTION OF V. INAEQUALIS RESISTANT TO BENOMYL IN APPLE

ORCHARDS IN ISRAEL

MATERIALS AND METHODS

Media. PDA + Chloramphenicol (250 µg/ml) + benomyl. In the preliminary test 0.5 µg/ml of benomyl were used. Higher concentration of benomyl (5 or 50 µg/ml) were added to the media for determination of resistance levels.

Preliminary examination of scab lesions. Leaves and fruit infected with scab were routinely collected from apple orchards during the years 1979-1981. From heavily infected orchards 100 scab lesions were tested by sampling two leaves from each of 50 trees. If the level of scab infection in an orchard was low, the maximum number (not more than 100) of available scab lesions were collected. Spores for the resistance test were removed from tagged and numbered scab lesions by placing a few drops of water onto a lesion using a pipette, and then withdrawing the suspension and placing it on the media (PDA alone and PDA + 0.5 µg/ml benomyl). Twenty-five samples were tested on 90 mm Petri dishes. After incubation for 48 h. at 8°C, spore germination was examined. Altogether the populations of 34 apple orchards and from various apple cultivars were monitored over three seasons 1979-1981.

Characterization of the resistant isolates. In the preliminary test, when spores were obtained from the scab-infected leaf or fruit, many contaminants were transferred to the test media. By tagging and numbering the lesions it was possible to further examine single spores from certain lesions. Pieces from 10 to 20-day-old single spore cultures were transferred to media supplemented with 0, 0.5, 5 and 50 µg/ml benomyl for further resistance tests (for details see 3,7).

Resistance stability was determined by examining 10 successive transfers of single-spore cultures on fungicide-free PDA, and then on

PDA + benomyl.

Single spore isolates were tested for pathogenicity by inoculating young leaves of a compatible apple cultivar. Resistance was tested in vivo by spraying the young leaves with 150 and 600 µg/ml of benomyl 24 hours prior to inoculation.

RESULTS

In the survey of apple orchards during the three seasons from 1979 to 1981, 1310 scab lesions from 40 orchards were found to be sensitive to benomyl. In the autumn of 1981 resistance to benomyl was found in seven orchards, all of them located in one region - the Golan Heights. In one of them, at Or-Tal, which was four years old in 1981, the scab population was monitored. Scab was found there for the first time in 1980. In 1981, before benomyl-resistance had been detected, the orchard received four fungicidal spray applications for scab-control, two of them with benomyl. Four infected rows (30 trees per row) of the scab-sensitive cv. Starking were selected for studying benomyl resistance. Initially, two resistant lesions were detected among the first 100 leaves sampled. Further samples from this plot revealed 2.3% resistance (22). In these Starking trees, 15% of the leaves were infected with scab on 15 October 1981. Single-spore cultures from 14 resistant scab lesions were used to determine in vitro the level of benomyl resistance. Isolates with three levels of resistance, MR, HR, and VHR (7), were identified. The source of the leaves infected with these benomyl-resistant isolates was traced to a few trees scattered in the plot. Thus, by sampling scab lesions from single trees over a relatively small area, isolates at three levels of resistance were obtained. Resistant V. inaequalis from the other seven orchards in the Golan Heights and from two orchards of cv. Anna on the Coastal Plain again revealed isolates with different levels of resistance. Scab lesions sampled from

these orchards during the following two seasons (1982, 1983) revealed isolates at four resistance levels, LR, MR, HR and VHR (21,22).

b. VENTURIA INAEQUALIS RESISTANCE IN NEW YORK

Using the monitoring technique previously described (25), V. inaequalis was obtained in Western New York from wild trees and from trees in abandoned orchards, and evaluated for resistance to dodine and benomyl. The results indicate dispersal of the resistant strains to many trees, as well as their survival in nature. The monitoring technique (25) has also been used to sample V. inaequalis lesions in an orchard (Poray) in which resistance to dodine had developed 11 years ago, and in an orchard where resistance to benomyl had developed three years ago. In both orchards, the fungicides to which resistance developed were not used during the intervening years. However, on the basis of results with the monitoring technique described, it is apparent that the resistant strains survived for three and 11 years in the absence of benomyl and dodine, respectively. In 1979 11 orchards, including abandoned orchards, were screened. In four of them benomyl-resistant isolates were detected (Richardson, Pellyzi, Lormont, Dunham). In 1980 benomyl-resistant isolates were obtained from five orchards, three of which contained isolates with double resistance, i.e., to dodine and to benomyl (Richardson, Syron and Preston). In four of them, mixed benomyl-resistant populations were identified: some of the isolates were LR and some were able to grow on 5 $\mu\text{g/ml}$ of benomyl. In 1980, spores of scab lesions from 21 trees were tested on 0, 0.5 and 5 $\mu\text{g/ml}$ benomyl. Of a total of 55 lesions, 9% were S, 62% were LR (0.5) and 29% were able to grow on 5 $\mu\text{g/ml}$ benomyl. In 1981 similar results were obtained: 16% were S, 52% were LR and 32% were able to grow on 5 $\mu\text{g/ml}$ benomyl. Single-spore isolates from Minns orchard were tested on 0, 0.5, 5 and 50 $\mu\text{g/ml}$ benomyl and found to be LR, HR and VHR (see MS. 3). The competitive ability of isolates resistant

or sensitive to benomyl was tested on apple seedlings. Two resistant isolates, one LR and the other VHR, did not differ in their pathogenicity and caused typical scab lesions on seedlings treated with 150 µg/ml benomyl. The fitness of LR isolate in a mixed population of 5% R + 95% S was tested on apple seedlings. After four successive inoculations the ratio of R to S did not change on untreated or on captan-treated plants. In plants treated with benomyl or benomyl + captan, the R isolate became the majority (19).

c. PATHOGENIC SPECIALIZATION IN V. INAEQUALIS

Leaves and fruit infected with scab were collected from apples in Israel. Six different cultivars were used to determine pathogenic specialization in V. inaequalis populations. Buds from six cultivars were grafted on root-stocks and cultivated in pots in a greenhouse. The six cultivars were: Red Delicious, Golden Delicious, Grand, Granny Smith, Anna and Hashabi (5,18).

Trees infected with the Hashabi pathotype were used for early detection of benomyl resistance. During the years 1979-1983, two scab-infected Hashabi trees in Neve Ya'ar and Ein Shemer were sprayed with 150 µg/ml of benomyl twice a year, once in the spring after detection of the first scab symptom and once in the fall. Samples of scab-infected leaves were routinely collected from the benomyl-treated Hashabi trees and from untreated trees, and tested for resistance on 0.5 µg/ml benomyl (18). Benomyl-resistant isolates were not detected in the population of the Hashabi pathotype V. inaequalis, despite its exposure to benomyl for four seasons.

DISCUSSION

In both Israel and New York benomyl has been included in the chemical control program, but has not been used as extensively as for scab control in pears. Resistance to benomyl in V. inaequalis appeared in New York after three years and in Israel after eight years of use. Confirmation of benomyl resistance in apple scab is still confined to relatively few orchards in

both countries. No crop losses due to benomyl resistance have been reported. It seems that the early detection of benomyl-resistant isolates in Western New York and in Israel enabled apple growers to use fungicides other than benzimidazoles for scab control. Effective replacements for dodine and benomyl were tested. Captafol (29) and EBI fungicides (5,30) were found to be highly effective. Bitertanol (Baycor) and etaconazole + captan (Sonax-captan) are recommended for apple growers in Israel. EBI fungicides were tested in a post-infection control program and found to be effective. V. inaequalis from plots (cv. Anna) treated with captafol-benomyl sprays in Israel (29) was monitored for benomyl resistance and found to be sensitive. The biological data obtained in the present research were used to prevent losses, and formed the basis of scab-control recommendations to the growers.

Our genetic research revealed that benomyl resistance had originated with independent mutations in a Mendelian gene. The availability of two collections of benomyl-resistant isolates, from New York and from Israel, enabled us to study and compare the inheritance of benomyl-resistance in V. inaequalis. In spite of their geographic remoteness, the same gene had mutated in both countries. From our results it is clear that the various levels of resistance were governed by different alleles of a single gene. Because of this allelism, the high level of resistance is unlikely to develop from interactions between low-resistance genes. The fact that MR, HR and VHR isolates were obtained in Israel from a few trees in a young orchard with a very short scab history (only two year) and with a low (~2%) frequency of resistant isolates supports the conclusion that the different alleles had originated in random and independent mutational events. Similarly LR, HR and VHR isolates were obtained from Minns orchard in New York.

The fitness research on seedlings in the greenhouse showed that an LR and a VHR isolate from New York were able to infect plants sprayed with

benomyl. Similar results were obtained in Israel after inoculating potted apple trees with MR, HR and VHR isolates. Although a LR isolate from New York was mixed with a S isolate in the ratio of 1:19, it was able to survive even after four successive inoculations on untreated plants. Moreover, the LR isolate survived even under a fungicide control regime in which the plants were sprayed with captan. It became a majority in the population when the plants were sprayed with benomyl or a mixture of benomyl and captan. Similar results on the fitness of the resistant isolates were obtained in the Or-Tal orchard in Israel, where benomyl resistance was detected in 1981. Although alternating spray applications of benomyl and captan effectively controlled scab during the following season (1982), a few infected leaves were detected in the fall. The frequency of benomyl-resistant isolates increased from 2.3% (in 1981) to 63% (in 1982). Of 512 single-spore isolates from 55 resistant scab lesions, 12.7% were MR, 34.6% were HR and 52.7% were VHR. These results show that benomyl-resistant biotypes are able to survive and persist in the orchard, even while the disease is being controlled by other fungicides. The fact that isolates with different levels of resistance were detectable in this orchard and in other apple orchards in Israel and in New York indicates that their fitness is similar and that there is no gradual shift from LR to VHR isolates in the benomyl-resistant population of V. inaequalis.

B. Venturia pirina

INTRODUCTION

Benomyl-resistant V. pirina has caused heavy losses in pear orchards in Israel. Resistance of V. pirina to benomyl was found in 1975, and during the next few years resistant isolates were evident in most of the pear orchards in Israel. The resistant isolates were stable and pathogenic. Genetic analysis showed that resistance to benzimidazole fungicides was controlled by a single Mendelian gene. Since 1976, benzimidazole fungicides have not been used for pear scab control.

The objectives of our research were:

To determine the fitness of the benomyl-resistant isolates (for details see 6);

To study the persistence of resistance in pear orchards in the absence of benzimidazole fungicides (for details see pp. 21);

To enable early detection of resistance in races I, III, IV and V. of V. pirina (for details see pp. 22 and Abstr. 18);

To find effective replacements for benomyl and thiophanate methyl in the control of pear scab (for details see 5,31).

a. PERSISTENCE OF BENOMYL-RESISTANT V. PIRINA IN PEAR ORCHARDS

During the years 1979-1983 scab-infected pear orchards were routinely inspected and V. pirina was monitored. V. pirina populations from two orchards which had been 100% resistant in 1975 (Tel Zoffit) and 1976 (Daliya) were still found to be 100% resistant after six years. From 1977 to 1983 no benzimidazole fungicides were used in these or in any other pear orchards. Resistant V. pirina was obtained from all the pear orchards sampled. While different frequencies of resistance were observed in these orchards, the level of resistance was the same - as in 1975, all the isolates were YHR.

b. EARLY DETECTION OF RESISTANCE IN RACES I, II, IV and V of V. PIRINA

Race V of V. pirina and its exclusive host, the wild pear Pyrus syriaca, were used. During the years 1979-1983, four scab-infected trees in four locations were sprayed with 150 ug/ml benomyl twice per season; once in the spring after the detection of early scab and once in the fall. Similar sprays were applied to trees infected with race I and race IV. Routine testing of samples of scab lesions from benomyl-treated and from untreated trees revealed sensitive V. pirina, although the populations of races I, IV and V were exposed to benomyl (18).

DISCUSSION

The results of our research on the fitness of the resistant isolates show that in the absence of selection pressure by the fungicide they were at least as pathogenic as the sensitive isolates. Research on the persistence of the resistant isolates in the pear orchard indicated that they were able to compete successfully with the sensitive population under natural conditions. In fact, even after a six year absence of benzimidazole fungicides, the resistant population remained stable. In two orchards the resistance status remained the same as it was when first discovered - 100% benomyl resistance. In orchards where the R isolates were a minority in V. pirina population they were still detectable even after six years. We may conclude that in the case of benomyl resistance and pear scab the resistance is well established and becomes a part of the V. pirina population. Thus, there is little chance that benomyl could be used for pear scab control. EBI fungicides have been found effective in pear scab control (5,31). Since 1982, bitertanol (Baycor) and etaconazole + captan (Sonax-captan) have been recommended for pear scab control in Israel.

5. EVALUATION OF THE RESEARCH AND ITS BENEFIT TO AGRICULTURE

The research described in this report was expected to yield the knowledge and provide the means of extending the life span of site-specific systemic fungicides for better disease management. The primary activities were:

- a. Determination of the factors involved in the spread of resistant strains in areas where resistance to benomyl has been already detected, i.e., M. fructicola in California and New York, V. inaequalis in New York, and V. pirina in Israel.
- b. Establishment of the means of preventing or delaying the development of fungicide resistance in areas where resistance to benomyl has not been detected, i.e., M. laxa and V. inaequalis in California and in Israel.

Evaluation of the research conducted according to the original proposal indicates that although the time schedule was followed, it was not possible to complete all of the work within the 3-year time period and with the funds granted.

Israel and New York

In both countries, crop losses due to scab control failures were avoided. For growers, the immediate benefit of our research was in the early detection of fungicide resistance. In New York, during the first year (1980), we detected isolates of V. inaequalis with double resistance to dodine and benomyl. This valuable information was followed by recommendations to apple growers on how to avoid scab control failures resulting from benomyl resistance in V. inaequalis. Similar benefit was derived by growers in Israel (in 1981) when V. inaequalis resistance was detected before it could cause apple crop losses.

Results from field tests obtained in the first year yielded data, extremely useful to growers, on replacements for benomyl in the control of brown rot and scab. Laboratory findings pointed to the risks involved in the exclusive use of EBI and dicarboximide fungicides for disease control.

The genetic and fitness research showed that the resistant isolates, even those in which resistance was low, are able to compete with the sensitive isolates that represent the original population of the pathogen before the selection pressure of the benomyl has started. More importantly, once resistance has developed the subsequent use of benzimidazole fungicide is ineffective, at least in the case of apple and pear scab. The findings that we were able to distinguish in vitro between isolates of V. inaequalis with different levels of resistance and could prove that the levels are inherited, stable, and of independent origin has important implications for the study of resistance of plant pathogens to fungicides.

California

The project was initiated upon the arrival of Dr. Ezra Shabi at the Department of Plant Pathology at the University of California, Davis. Methods were established for determination of the baseline sensitivity of Monilinia spp. to benomyl, and for characterization of Israeli and California isolates of the pathogens. Field plots were jointly established.

Excellent results in field tests were obtained in the second year. Monitoring programs provided the data required to decide critical issues on the benefits of combination treatments of benomyl with another contact fungicide for control of brown rot. Fitness tests comparing the benomyl-resistant and benomyl-sensitive lines in the laboratory indicated that the benomyl-resistant lines were highly competitive in the orchard, although some trend towards reduced competitiveness was indicated in in vitro and in vivo tests. In order to study the possible spread of the benomyl-resistant lines from one location to another, barrage tests were conducted

using a number of isolates, both benomyl-resistant and benomyl-sensitive. We hoped to find orchards with one or two barrage groups, but many barrage groups were found. Apothecia were found which contained both benomyl-resistant and benomyl-sensitive ascospores, adding further to the number of barrage groups. In genetic studies, fruits inoculated with various combinations of benomyl-sensitive and benomyl-resistant lines were buried in an experimental orchard and in an orchard where apothecia were produced naturally. During the first year no apothecia developed on the inoculated fruit, but in the second year one apothecium was found on fruit placed in the experimental orchard. Studies are in progress to determine whether the apothecium originated from our inoculated fruit.

Benomyl-resistant M. laxa was detected and studies were carried out to characterize the resistant isolate collected from apricots. The resistant isolate appeared less fit. Benomyl-resistant M. laxa was also found in almond orchards. The differences in fitness between the apricot and the almond isolates are being studied under a grant from the Almond Board of California.

During the third year we established the benefits of the sterol-inhibiting fungicide, triforine, for control of both M. laxa and M. fructicola. Iprodione was tested as another possible alternative to benomyl. Triforine-resistant M. fructicola was not detected in orchards where triforine treatments failed to control the disease. According to those present at the 4th International Congress of Plant Pathology (August 1983), resistance to sterol-inhibiting fungicides such as triforine is not expected to develop under field conditions. Manuscripts finalizing the project have been completed and no further manuscripts are planned with the current partial support from BARD.

The benefits from this project have extended not only to the USA and Israel but also to Brazil, where benomyl-resistant isolates have not caused serious losses to the stone fruit industry. Cooperation with researchers in Brazil has made possible the development of another essential component in

disease control: host resistance. One cultivar of peach grown commercially in Brazil was found to be resistant to Monilinia fructicola (26), and a cooperative project is being established between Brazil and USA.

Aspects of the research that were not completed were determination of the parameters which influence spread of the benomyl-resistant Monilinia, and critical examination of the differences in characteristics between artificially produced benomyl-resistant lines of Monilinia and lines which occur naturally within the orchard.

6. DESCRIPTION AND RESULTS OF COOPERATION

The project collaborators were able to meet several times to discuss the work. In addition, Dr. E. Shabi spent the first year of the project (six months) with Dr. J.M. Ogawa at the Department of Plant Pathology, University of California, Davis, and three months with Dr. J.D. Gilpatrick at the Department of Plant Pathology, New York State Agricultural Experiment Station, Geneva, Cornell University, New York. During these visits joint research, discussions, and coordination of the research plans were fruitful and beneficial. Ogawa and Gilpatrick participated in a conference on Pest Resistance to Pesticides; Challenges and Prospects at Palm Springs, California on 3-7 December 1979. Gilpatrick, Ogawa and Shabi participated in the symposium on Resistance to Fungicides in Plant Pathogens at Wageningen on 3-6 August 1981. At this symposium, six papers were presented by participants in this BARD project (13,14,17,18,19,23). During these meetings fruitful discussions were held and there was considerable exchange of ideas and information on the various activities carried out in the context of the BARD research.

Cooperation between the three researchers was of benefit to both countries, Israel and USA. The improved ability to detect early resistance in M. laxa in California and in V. inaequalis in Israel made it possible to avoid crop losses in California, Israel and New York. From the research results in California, the Israeli participant worked out a strategy for avoiding or delaying resistance of M. laxa in Israel. Research on laboratory resistant isolates in New York (M. fructicola to EBI fungicides) and in Israel (M. laxa to dicarboximide fungicides) was extremely valuable to the fruit industries in the USA and Israel, because it allows predictions of the risk that fungicides will lose their efficacy because of resistance. The cooperative research of Gilpatrick and Shabi on the fitness and genetics of V. inaequalis isolates yielded some ideas on how to cope with this threat

to agriculture. The most important achievement of this work lies in the fact that it is the first international research project aimed at studying the resistance of plant pathogens to fungicides. No research project of this nature has previously been undertaken for almost four seasons in three countries. The cooperative framework provided the research participants with the opportunity to examine their ideas on how to cope with a pressing and urgent problem within three different agricultural communities.

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