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Resistance to fungicides in plant pathogens: abstracts of papers

Symposium organized on the occasion of the 75th Anniversary of the Laboratory of Phytopathology, Wageningen, the Netherlands, 3-6 August 1981

Seventy-five years of phytopathology in Wageningen

J. DEKKER (Laboratory of Phytopathology, Agricultural University, Wageningen, the Netherlands)

In 1906, the Institute of Phytopathology was established at Wageningen as part of the State Agricultural School, which later on, in 1918, was elevated to university level. The first director was Jan Ritzema Bos, who in addition was editor-in-chief of the in 1891 established 'Tijdschrift over Plantenziekten' (now 'Netherlands Journal of Plant Pathology') and director of the Plant Protection Service. In 1919, Ritzema Bos was succeeded as director of the Institute by H. M. Quanjer, who has become known for his work on virus diseases of potatoes. After his retirement he was succeeded by A. J. P. Oort, who stimulated various new developments in plant pathology, among others the search for systemic fungicides.

Nowadays, in Wageningen research on plant diseases and pests is carried out at departments of the Agricultural University (Entomology, Nematology, Phytopathology and Virology), the Research Institute for Plant Protection (IPO) and the Plant Protection Service (PD).

Thirty years Research Unit Internal Therapy of Plants

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In 1951, the Research Unit for Internal Therapy of Plants, TNO was established, on the initiative of A. J. P. Oort, then director of the Laboratory of Phytopathology at Wageningen, and of G. J. M. van der Kerk, then director of the Institute for Organic Chemistry, TNO at Utrecht. In this research unit a close co-operation exists between scientists of different disciplines: plant pathology, plant physiology, organic chemistry, and microbiology.

Up to about 1969, the full emphasis was on the search for systemic fungicides, suitable for plant disease control. To this end, new compounds were synthesized and methods designed for the screening of these compounds for systemic control of fungal diseases. After systemic fungicides had become available for practical application, the emphasis in the work shifted towards studies on the mechanisms of action of fungicides, and on the development of fungicides resistance in plant pathogens, the topic of this symposium.

What can be learned from nearly 40 years of development of insecticide resistance

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Resistance mechanisms have been studied intensively and are now known to be mainly dependent on a variety of detoxication mechanisms, or on alterations at the site of action. The former are modifications of a series of mechanisms attacking 'natural compounds', which can even be adapted to degrade very tough substances such as DDT and DDE. Sites of action can alter in the reaction with insecticides without serious impairment of the related reaction with natural substrate. To many insecticides several kinds of resistance have developed. Certain resistance mechanisms can be overcome by suitable alternative insecticides, by blocking detoxication with synergists, or by development of 'negatively correlated' analogues of the insecticides only affecting the altered, resistant, site of action. Development of new resistance mechanisms can often incapacitate such measures.

Resistance genes are sometimes extremely rare, but once selected can be quite persistent in the absence of selection. Generally, resistance is brought about by one or a few genes. Whether these are single-step mutations has never been established.

Cross-resistance is one of the most serious problems. Not only can it cause resistance to groups of related insecticides (in particular if the site of action is altered), but also to quite unrelated compounds, since common factors such as detoxication or penetration can affect widely different chemicals. In populations subjected to many insecticides, multiple resistance can develop and cross-resistance can maintain and even further increase resistance to compounds no longer used.

Prediction of resistance to insecticides is hardly possible, because of the enormous variation in the development of resistance in different species. Experimental assessment of the chances of resistance development is difficult, because of the rarity of the genes involved, and because polygenic adaptation may develop in the laboratory but not in the field.

Variation amongst species in rate and extent of resistance development is enormous, as is the variation between insecticides. Despite serious resistance problems, insecticides still constitute the main way of controlling insects, and their replacement by other methods, although studied intensively, is only slow. The most likely candidates for successful control are probably viruses and bacterial agents.

Strategies to avoid resistance, other than restricted use of insecticides in time and space, are being studied and models to utilize different compounds in checkerboard patterns have been proposed. Their success has yet to be proved. The same applies to release of susceptible insects for the reduction of resistance. That strategy has to overcome the disadvantage of costly breeding, distribution and surveying facilities, a particular problem in tropical areas.

Obvious differences from fungicide resistance are the much greater impact of detoxication in animals than in plants, and the much larger numbers of individuals in fungi, in particular of spores. The larger number increases the chances of rare mutations being selected for and facilitates an experimental approach for testing the likelihood of development of resistance.

A review of methods used to assess the sensitivity of fungi to fungicides

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In this review attention is focussed on some of the basic concepts which affect the collection and interpretation of data.

A study of the sensitivity of a fungus to a fungicide is essentially a study of the changes in the dose response of the population which occur under the influence of the fungicide. The methods used must therefore be designed to sample the relevant populations, to measure changes in dose response, and to relate these to the overall population of the fungus.

Methods of sampling the population, culturing the fungus, applying chemicals and inoculating test units will be considered, together with some of the hazards of interpreting the data.

Variation in two barley pathogens towards systemic fungicides

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The variation within natural populations of barley powdery mildew *(Erysiphe graminis* f. sp. *hordei)* towards three systemic fungicides was examined using laboratory assays. Variation towards tridemorph was limited and hardly altered following Calixin treatment. Although significant variation to triadimenol was not encountered at high doses [LD_{95}], at lower doses [LD_{20}] considerable variation existed. At these doses some genotypes were not affected by triadimenol, and their increase in populations exposed to Baytan (or Bayleton), led to changes which lasted several months. Most variation occurred towards ethirimol, and genotypes able to infect barley grown from Milstem treated seed were readily identified. Milstem increased their frequency and reduced the overall range of variation, but these shifts were short-lived and populations soon resembled those on untreated crops. *In vitro* assays have also identified variation towards triadimenol in barley leaf scald *(Rhynchosporium secalis).* Its significance, along with cross-resistance patterns in both mildew and scald will be discussed.

Mutant of Ustilago maydis genetically blocked in sterol C-14 demethylation

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A sterol C-14 demethylation deficient mutant of *Ustilago maydis* was selected on amphotericin B containing medium. The mutant sporidia lack ergosterol but contain 24-methylenedihydrolanosterol, obtusifoliol and 14α-methylfecosterol as major sterols. The same three sterols accumulate in wild type *U. maydis* sporidia treated with triarimol, fenarimol, miconazole or etaconazole.

Doubling time of the mutant is about 6.5 h compared to about 2.25 h for the wild type. At this rate of doubling, projected cell yield of the mutant after 18 h is only about 5 percent of that of the wild type.

Tests carried out on agar medium indicated that normal growth rate is not restored in the mutant by exogenous ergosterol. Morphologically, mutant sporidia are abnormal in comparison with those of the wild type. Many of the sporidia are enlarged, branched and multicelled. They resemble sporidia of the wild type treated with 2 μg/ml of fenarimol. Free fatty acid content of the mutant sporidia is appreciably higher than that of wild type sporidia. The increased free-fatty acid content resembles that found in wild type sporidia treated with triarimol, fenarimol, miconazole or etaconazole.

The mutant is appreciably less sensitive than the wild type to fenarimol and miconazole. The ED_{50} values of fenarimol for the wild type and mutant at an initial inoculum of approximately 0.1 mg cell dry wt per ml, are 0.25 and greater than 10 μg/ml respectively. Corresponding ED_{50} values of miconazole for the 2 strains are 0.02 and 1.1 μg/ml. There is no inhibition of growth of the mutant by up to 2.5 μg/ml of fenarimol or by up to 0.5 μg/ml of miconazole. Growth of the mutant is inhibited only about 25 percent by 10 μg/ml of fenarimol but is strongly inhibited by concentrations of miconazole of 2.5 μg/ml or greater.

This study indicates that a) the primary toxicity of fenarimol and miconazole in *U. maydis* is based on inhibition of sterol C-14 demethylation and b) there is also one or more sites of lower sensitivity to each of these fungicides in this organism. The sites of secondary sensitivity may sometimes play a role in the practical performance of miconazole, but it is doubtful that they are important in the case of fenarimol.

Implications of the stereochemistry of ergosterol biosynthesis inhibitors, especially with regard to resistance

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Due to the presence of an asymmetric carbon atom most of the ergosterol biosynthesis-inhibiting fungicides are chiral compounds. Such compounds exist in two different stereoisomeric forms that are nonsuperimposable mirror images of each other, so-called enantiomers. Compounds with two or more asymmetric carbon (or other) atoms show an additional form of stereoisomerism, viz. diastereo(iso)merism. With two chiral centra (as f.i. in triadimenol), a compound occurs in four stereoisomeric forms, that means in two chemically slightly different diastereomers, each consisting of a pair of enantiomers.

Up to now, the implications of stereoisomerism, especially of enantiomerism, though widely recognized in other areas, were largely neglected in the field of (agricultural) fungicides. However, in insecticide research, especially of the natural and synthetic pyrethroids, and in particular in insect pheromone research, stereochemistry has become a matter of keen interest. For instance, studies on 'stereochemistry - pheromone activity relationships' showed that of the two enantiomers of a pair a) both can be active, b) both are required for activity, or c) only one is active, the

other being either indifferent or even inhibitory. Also in pharmacology stereoselective effects of enantiomers and diastereomers are widely known. Differences in activity of enantiomers and diastereomers have been ascribed to a series of factors such as a) differential uptake, b) differential intrinsic activity at the target site (due to specificity in drug-receptor interactions), c) differential metabolic conversion or degradation, d) differential rate of metabolism, etc.

Especially studies on so-called congeneric series of compounds have contributed considerably to present-day knowledge on the biological implications of stereochemistry; their results are nowadays taken into consideration by controlling drug geometry in drug design. An important unifying principle in stereochemistry has been discovered by Ariëns and coworkers (cf. Lehmann F., P.A. et al., 1976. In: E. Junker (Ed.), Progress Research. Birkhäuser Verlag, Basel/Stuttgart, Vol. 20, p. 101-142): direct comparison of the isomeric activity ratio (A/B) with the activity (A) of the most potent member of each pair of enantiomers in such a congeneric series (in which B denotes the activity of the lesser active member) led them to conclude, that log A/B is a linear function of log A. When the slope of this function is zero, the chiral center of the enantiomers is not critically involved in the binding of the enantiomers to the target site or, in other words, chirality is non-critical to stereoselectivity. That means that in such cases the asymmetric center does not play a selective role in the differential activity of the two members of the enantiomeric pair. In other instances, however, stereoselectivity wholly or partially depends on chirality. Among epimeric diastereomers, with two chiral centers, it is even possible that one of these is critically and the other non-critically involved in the interaction with the receptor.

Two congeneric series of ergosterol biosynthesis inhibitors, viz. the imidazoles and the triazoles, seem to present outstanding 'tools' to study the effects of stereoselectivity in the field of agricultural fungicides; in addition, the morpholines and the pyrimidines might present other examples to examine these phenomena, which might provide a better insight into the lack of cross-resistance between structurally related fungicides and the mechanisms of resistance as well. Further, increasing knowledge about the effects of the formulation used on the ratio in which both stereoisomers are present and their fungitoxic activity, as well as about differential metabolism of stereoisomers seems a prerequisite to fully comprehend the significance of stereoisomerism in the mode of action of ergosterol biosynthesis inhibitors.

The extensive study on differential intrinsic activity and metabolism of the two diastereomers of triadimenol by Buchenauer and Grossmann (Z. PflKrh. PflSch. 88 (1981) in press) already suggests, that a more rational development of such new fungicides with optimized bioactivity to a wide range of fungi will be a formidable task. Perhaps, the use of chiral synthons, such as microbially produced epoxyalkanes, as envisaged in the field of biotechnology, will help to produce the proper fungicides.

Manipulation of fenarimol uptake by Aspergillus nidulans

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Uptake of fenarimol by the wild-type strain 003 of *Aspergillus nidulans* is characterized by a rapid initial accumulation, followed by a gradual release of the fungicide. Rapid accumulation is due to passive influx. Release of the fungicide is ascribed to an energy-dependent efflux mechanism, which is activated by the fungicide itself and reaches its maximum activity after about 60-90 min of incubation. As a consequence uptake attains a low level which then remains constant due to an equilibrium between influx and efflux. Uptake of fenarimol by fenarimol-resistant strains is always low and constant in time. This is caused by a high energy-dependent efflux activity which is constitutive and thus does not need to be activated.

Respiratory inhibitors (f.i. CCCP) inhibit energy-dependent efflux activity. Hence, they effect a high level of fenarimol uptake in both wild-type and resistant strains. This is probably the cause of the synergistic effect of some of these inhibitors on toxicity of fenarimol to both strains as revealed by crossed-paper strip bioassays. Some conventional fungicides like the phthalimides (f.i. captafol) have a similar action as these respiratory inhibitors. Therefore, conventional fungicides may have potential significance in synergizing toxicity of sterol-biosynthesis inhibitors to wild-type and resistant strains of plant pathogens.

Other sterol-biosynthesis inhibiting fungicides (imidazoles, morpholines, pyrimidines, and triazoles) also activate the energy-dependent efflux of fenarimol in the wild-type strains of *A. nidulans*. As a consequence, the uptake of fenarimol by the fungus is low. This is probably the reason for the antagonism of these fungicides on toxicity of fenarimol in crossed-paper strip bioassays when the fenarimol strip is applied 10 h after application of the strip with the test fungicide. Upon successive application, sterol-biosynthesis inhibiting fungicides might therefore in theory interfere with each other in their efficacy against plant pathogens.

In conclusion, uptake of fenarimol by wild-type and fenarimol-resistant strains of *A. nidulans* can be manipulated by interfering with an energy-dependent efflux mechanism for fenarimol. Interference with the mechanism may influence the effectiveness of fenarimol. Circumstantial evidence suggests that ATPase-related membrane processes are involved in the efflux of the fungicide.

Inhibition of endopolygalacturonase from Geotrichum candidum by CGA 64251 (Sonax WP 10%) and its possible implication for resistance development

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The experimental fungicide Sonax (CGA 64251 WP 10%) at a level of 500 μg/ml, significantly prevented the expansion of sour rot-infected areas on lemon fruits when applied within 8 h after inoculation. A homogeneous preparation of endopolygalacturonase from *Geotrichum candidum* was inhibited by approximately 80% in

the presence of 50 μg/ml Sonax. The inhibition was noncompetitive. An irreversible and complete inhibition of endopolygalacturonase bound to the surface of *G. candidum* spores was obtained within 10 min at a Sonax concentration of 250 μg/ml.

Sonax also caused inhibition of enzyme-dependent maceration of albedo tissue from lemon peel. The inhibitory effect of Sonax was apparently associated with the formulation of the fungicide and not with its active ingredient.

The sensitivity of populations of Erysiphe graminis to triadimefon and Botrytis cinerea to iprodione

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In 1979 and 1980, surveys were made in the North of England of the sensitivity of populations of *Erysiphe graminis* in spring barley crops. A mobile nursery technique was used in which cv. Golden Promise seedlings treated at 0.025, 0.05, 0.1, 0.25, 0.5, 1.0 and 2.5 g Bayleton (25% triadimefon) per kg of seed, were exposed in crops for 4-6 days and the mildew assessed after at least a further 9 days. The sensitivity of a population was measured as either the sensitivity score (seedlings from the highest fungicide seed treatment on which mildew occurred) or the FD_{50} value (seedlings from the highest fungicide seed treatment with half the foliar disease level of the untreated control).

In 1979 mildew levels in crops were low and many were not fungicide treated. Of the 113 populations examined 63% showed a sensitivity score of 0.05 or less. In 1980 mildew was severe and most crops were fungicide treated. 173 populations were examined and 56% had sensitivity scores of 1.0 or above. FD_{50} measurements were not possible in 1979 because of the low incidence of mildew but in 1980, 43% had an FD_{50} value of 0.05 or less and 7% 1.0 or more. There was no apparent disease control failure in any of the crops examined.

In 1980, 804 isolates of *Botrytis cinerea,* collected from various crops and locations, were examined for their sensitivity to iprodione and benomyl, by plating mass mycelial transfers on to potato dextrose agar amended with 2 or 20 μg/ml iprodione or 20 μg/ml benomyl, respectively. Only 0.6% of these isolates grew at 2 μg/ml iprodione and none at 20 μg/ml whereas 37% grew at 20 μg/ml benomyl. The iprodione insensitive isolates were from lettuce (2), cyclamen (2) and pelargonium (1). All showed reduced virulence in a pathogenicity test but in the presence of iprodione were able to produce disease. All iprodione insensitive isolates were also insensitive to benomyl.

Insensitivity of Erysiphe graminis f. sp. hordei to triadimefon

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In 1980, isolates of *Erysiphe graminis* f. sp. *hordei* from northern England showed considerable insensitivity to triadimefon. Consequently, in 1981, a spore-trap mount-

ed on a car roof and containing barley and wheat seedlings, treated or not with triadimenol, was driven along particular routes in eastern England, to determine the general frequency of insensitivity in the atmosphere, and any change of frequency with time. Mobile nurseries containing similar seedlings were exposed in barley and wheat crops to determine the occurrence and extent of insensitivity in individual populations. The field performance of plots of a mildew-resistant and a mildew-susceptible barley variety, treated or not with triadimenol, was also observed.

Preliminary results indicate that insensitivity to triadimefon and triadimenol is relatively common, less so in *E. graminis* f. sp. *tritici* than in *E. graminis* f. sp. *hordei*. In the latter, there was an increase in the relative frequency of insensitivity during June. Evidence for insensitivity in field crops was strongest for some mobile nurseries exposed in spring barley sprayed with triadimefon.

This phenomenon is not yet known to be of practical significance, but continued widespread use of these and related fungicides will increase the risk. Some restriction is desirable: one method is to incorporate triadimenol-treated seed of three different varieties in a mixture with a larger proportion of untreated, mixed seed. This provides good disease control at low cost with reduced selection for insensitivity.

Resistance to ergosterol biosynthesis-inhibiting fungicides in laboratory strains of Monilinia fructicola

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Strains of *Monilinia fructicola* were selected from transfers which grew on PDA amended with Vanguard (CGA 64251), [1((2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2yl)methyl)-1*H*-1,2,4-triazole]. A sensitive (S) isolate exhibited about a 50% reduction of radial growth on agar amended with 0.1 μg/ml and was completely inhibited at 1.0 μg/ml, while the resistant (R) isolates grew at 1.0 μg/ml. *In vitro,* R isolates also were less sensitive than wild types to other ergosterol biosynthesis-inhibiting fungicides - prochloraz, bitertanol, fenarimol, and triforine. R isolates were as pathogenic to peach and apple fruit and cherry flowers as S isolates, and sporulated abundantly on them. However, significant differences in control of disease in these hosts were not observed when treated with Vanguard before inoculation with either R or S isolates. R isolates of peach were readily recovered in the spring from artificially infected (mummified) peach fruit after exposure outdoors during winter conditions of New York State.

Cross-resistance between ergosterol biosynthesis-inhibiting fungicides in Aspergillus nidulans, Botrytis cinerea, Penicillium expansum and Ustilago maydis

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Ergosterol biosynthesis-inhibiting fungicides cause either accumulation of C-4 (and C-14) methylated sterols or of 4-desmethyl sterols. Various triazole derivatives (di-

clobutrazol, propiconazol, triadimefon, triadimenol), imidazole derivatives (imazalil, phenapronil, prochloraz) and pyrimidine-carbinols (fenarimol, nuarimol [group I] which mainly block C-14 demethylation of ergosterol precursors have the first type of effect. In the second case there is probably an inhibition of $\Delta^8 \rightarrow \Delta^7$ isomerase because several Δ^8 sterols are detected in fungi treated with dodemorph, fenpropidin, fenpropimorph or tridemorph [group II].

Resistance to members of group I and II can be obtained in laboratory experiments with various fungi. The phenomenon of cross-resistance among these ergosterol biosynthesis inhibitors has been studied with strains of *Aspergillus nidulans, Botrytis cinerea, Penicillium expansum* and *Ustilago maydis.* With the three filamentous fungi, cross-resistance occurred always between the fungicides of each group (but not between members of group I and II). In *Ustilago maydis,* we observed tolerance to:

a. all the fungicides of group I
b. imidazoles and pyrimidine-carbinols but not to triazole derivatives
c. all the fungicides of group II
d. fenpropidin but not to the morpholin derivatives

Strains of types b and d were generally more resistant than those of types a and c. The sterol contents of sensitive and tolerant strains are similar.

Is there cross-resistance between ergosterol biosynthesis inhibitors and dicarboximides?

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Because of alleged interference with sterol biosynthesis by the dicarboximide fungicide iprodione, it was of interest to investigate whether strains of fungal species known to be resistant to ergosterol biosynthesis inhibitors displayed cross-resistance to dicarboximides. Widespread cross-resistance between these two different groups of fungicides would, indeed, be of practical consequence, and simultaneous or sequential use of representatives of both groups might mutually create potential hazards to the performance of those fungicides among them, to which resistance has not emerged so far.

Several strains of *Aspergillus nidulans, Cladosporium cucumerinum* and *Penicillium italicum* with known resistance to ergosterol biosynthesis inhibitors were tested, in mycelial growth tests and TLC-bioassays, for resistance to iprodione, procymidone and vinclozolin. Negligible to very low levels of resistance to iprodione only were observed in one out of three strains of *A. nidulans* and in two out of six strains of *P. italicum.* Two strains of *C. cucumerinum* displayed a low resistance to iprodione, and a high resistance to vinclozolin. The behaviour towards procymidone was somewhat anomalous, both in mycelial growth tests and in TLC-bioassays; the resistance to procymidone was moderate to high. Remarkably, the latter strains proved also to be moderately resistant to the isoflavonoid phytoalexins medicarpin and pisatin; they were, however, sensitive to pimaricin. The 'all-round' resistance of these *C. cucumerinum* strains was accompanied by a strongly reduced pathogenicity, thus annihilating their potential danger under practical conditions.

Resistance to dicarboximide fungicides in laboratory isolates of Monilinia laxa

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Monilinia laxa, the incitant of blossom blight in stone fruits in Israel, is sensitive to 3 μg/ml of the dicarboximide fungicides vinclozolin and iprodione in the growth medium. When a large number of spores, from an isolate never exposed to these fungicides, was seeded on a medium containing 15 μg/ml iprodione, spontaneous resistant mutants appeared at 10^{-5} frequency. These mutants showed cross-resistance to the dicarboximide fungicides vinclozolin and procymidone and were therefore designated VIP (= Vinclozolin/Iprodione/Procymidone)-resistant; they were also resistant to dicloran and to fungicides Co 4462 and Co 6054. Growth rate on fungicide-free medium was similar to that of the parental sensitive strain but the sporulation was reduced. Growth rate on media supplemented with dicarboximide fungicides decreased gradually with increasing fungicide concentrations. The VIP-resistance has been stable for more than one year in the absence of fungicides. Artificial inoculation of cherry, apricot, and plum fruits, previously treated with 0.1% of the formulated fungicides vinclozolin (Ronilan 50 WP), iprodione (Rovral 50 WP), procymidone (DPX 4424 50 WP), or Co 6054 (50 WP), with a VIP-resistant strain, resulted in brown rot, while similar treatment provided full protection of the fruits against the sensitive strain.

Resistance of Botrytis cinerea Pers. to dicarboximide fungicides

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Dicarboximide fungicides have been used for the control of gray mould in Crete since 1976. The last years a decrease of their efficacy was observed. In successive surveys carried out in May 1980, February 1981 and May 1981 in 28, 11 and 13 greenhouses, respectively, a considerable proportion of resistant strains was noted. Either resistant or sensitive strains were isolated from all the greenhouses sampled. In two greenhouses among three in which resistant strains were sampled, the resistant strains continued to be present during the whole sampling period. On the other hand, in one among five greenhouses in which only sensitive strains were present, resistance developed as revealed with the last survey. In three of the greenhouses with resistant strains there was an acute control problem. The ED_{50} of seventeen resistant strains studied was around 2.5 μg/ml vinclozolin. They were also resistant to procymidone, iprodione and dicloran. In most of the cases strains resistant to vinclozolin were also resistant to benomyl. The growth of the resistant strains on PDA was slower than the growth of the sensitive ones, but spores germinated equally well in both cases. Young egg-plants treated with 0.075 μg/ml of vinclozolin were easily infected when they were inoculated by a spore suspension of resistant strains.

Competition in vitro and in vivo between strains of Botrytis cinerea Pers. sensitive and resistant to dicarboximides

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Dicarboximide-resistant strains of *Botrytis cinerea* Pers., very easily obtained under laboratory conditions, have been observed in different countries on several crops. The competitiveness of resistant and sensitive parent strains of *B. cinerea* was evaluated *in vitro* and *in vivo*.

In vitro experiments were carried out by plating mixtures of sensitive and resistant conidia (50 : 50; 10 : 90) of the different strains on fungicide-free medium during four generation cycles and by determining, at each generation, the ratio of sensitive and resistant conidia. Generally the mixed cultures showed a disappearance of the resistant mutant. In some cases, after one generation all conidia produced by the mixture were sensitive to vinclozolin; with other strains, the percentage of resistant colonies decreased much more slowly. Only in one case, the resistant mutant was equally competitive with the sensitive one.

In vivo experiments were carried out on grapes and roses to compare the fitness of the resistant strains found to be most competitive *in vitro* to that of sensitive ones. After 2-3 months it was possible to reisolate the resistant strains from plants either sprayed or not with vinclozolin, but only at a very low frequency.

The fact that up to now resistance to dicarboximides was observed only in a few cases in the field can be partially explained by the generally lower saprophytic and pathogenic capacities of the most resistant strains *in vitro*. The risk for emergence of resistance in the field should not, however, be underestimated because of the possibility that resistant strains can also have biological and pathogenic qualities that make them fit to survive and compete.

Fitness of procymidone-resistant Botrytis cinerea strains developed in vitro

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Procymidone-resistant *Botrytis cinerea* spontaneously emerged *in vitro* from conidia and mycelia. When conidia of 74 isolates were respectively plated on an agar medium amended with 100 μg/ml procymidone, resistant strains were detected at a frequency ranging from $< 4 \times 10^{-9}$ to 21×10^{-6}. On the contrary, development of resistant strains was not experienced in a model experiment on diseased fruits of eggplant under the pressure of procymidone. Although resistant strains obtained *in vitro* differed considerably from each other in their characteristics, they all showed a lower ability to sporulate on agar media and to attack cucumber leaves than wild strains sensitive to procymidone.

To evaluate the relative potential of the resistant strains of *B. cinerea* to survive under natural conditions, sensitive and resistant strains were mixed-inoculated on rose plants or eggplants grown in a greenhouse. Resistant strains did not become do-

minant in a population on the plants and finally disappeared in the absence of selection pressure by procymidone. With procymidone applications, resistant populations increased gradually. However, withdrawal of the fungicide resulted in rapid decrease in resistant populations. Even in cases that the resistant population was high, the treatment of procymidone was still effective in controlling the disease.

Resistance to dicarboximide fungicides in Botrytis cinerea from cucumbers, tomatoes, strawberries and roses

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Wild types spores of *Botrytis cinerea* did not germinate, nor did mycelia grow, on media containing 5 μg/ml (a.i.) of dicarboximide fungicides (iprodione, vinclozolin, procymidone). Spores from diseased cucumbers and tomatoes, from greenhouses where Rovral and Ronilan failed to control gray mold, germinated on media containing 300 μg/ml of these fungicides, resulting in colonies carrying viable resistant spores. As much as 95-100% resistance was found among hundreds of samples from seven cucumber greenhouses in five sites. Incidence of resistant *B. cinerea* was also found in strawberries and roses.

Protection of plants against wild-type sensitive isolates, but not against dicarboximide-fungicide-resistant isolates of *B. cinerea,* was observed in the following *in vivo* systems, where plant parts were inoculated with mycelial plugs: (a) Detached leaves from cucumber plants previously sprayed with 0.1-0.5% formulated Rovral or Ronilan; (b) Intact cucumber plants drenched with DPX 4424; (c) Detached rose petals sprayed with 0.1-0.5% formulated Ronilan; (d) Cucumber seedlings sprayed with 0.2% formulated Rovral, Ronilan, Serinal, DPX 4424 or Co 4462. The dicarboximide-fungicide-resistant strains were only partly resistant to fungicide Co 6054 *in vitro* and *in vivo.*

Resistance to 3,5-dichlorophenyl-N-cyclic-imide fungicides

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The 3,5-dichlorophenyl-N-cyclic-imides (iprodione, procymidone, vinclozolin) are active against various plant pathogens, especially against *Botrytis cinerea*. Resistant strains of this fungus can be obtained in laboratory experiments or isolated from treated crops (strawberries and grapes). Their degree of tolerance towards cyclic-imide fungicides is either high (resistance factor greater than 1000) or low (resistance factor about 10). These strains exhibit cross-resistance to various aromatic compounds (acenaphthene, biphenyl, chloroneb, dichlobenil, dicloran, fluorene, pentachloroaniline, pentachlorobenzene, phenanthrene, *o*-phenylphenol, quintozene, tecnazene). They are also tolerant to methanol but not to ethanol, 1-propanol or 1-butanol. High levels of sugars (glucose, maltose, mannose, sucrose, xylose), polyols (mannitol, sorbitol) and salts (potassium or sodium chloride) are more toxic to

fungicide-resistant strains than to sensitive ones. All these results are also obtained with *Aspergillus nidulans, Penicillium expansum, Rhizopus nigricans* and *Ustilago maydis.*

On artificial medium or cucumber fruits, the moderately resistant strains of *Botrytis cinerea* generally present a good competitiveness whereas highly resistant ones have a reduced fitness. This phenomenon depends upon sugar concentrations in nutrient medium and level of total inoculum.

First practical experiences with metalaxyl resistance

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During 1980 and the first half of 1981, cases of metalaxyl failures in connection with the emergence of resistant strains of the target fungi have been observed on cucumber under plastic *(Pseudoperonospora cubensis)* in Israel, Greece and Japan; on potato *(Phytophthora infestans)* in some countries of Western Europe and South America and in New Zealand; on shaded tobacco *(Peronospora tabacina)* in Nicaragua and in nursery grapes *(Plasmopara viticola)* in South Africa. In all cases resistance was associated with the repeated and exclusive use of metalaxyl alone, while the use of mixtures with protective fungicides in geographically separate but epidemiologically comparable areas have not led to any resistance problems. Other common features were high disease pressure on susceptible cultivars and a tendency toward curative use. The resistance factors were generally above 100 × . No evidence for decreased fitness of resistant strains is available so far.

To prevent further cases of resistance Ciba-Geigy has adopted as a basic strategy the use of prepack mixtures of metalaxyl with protective fungicides against foliar *Oomycetes.* This mixture concept is judged preferable to alternations with single products or to tank mixtures, especially since such programmes are difficult to enforce at the grower level. In addition, prepacks offer the advantage of broader spectrum of control (secondary diseases), less damage risk, and in certain circumstances better control of *Oomycetes* than with acylalanines alone, particularly at the end of the season. In addition, soil treatments against foliar diseases are generally not recommended. Against soilborne *Oomycetes,* where the resistance risk is considered to be lower and where the options for mixture partners are very limited, granular and seed dressing formulations of metalaxyl alone are available.

Use of potato leaf discs for the measurement of metalaxyl sensitivity in isolates of Phytophthora infestans

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Discs of potato leaf tissue floated upon distilled water develop severe symptoms of blight when inoculated with droplets of a suspension of zoospores of *Phytophthora*

infestans. The development of this fungus examined so far was very severely restricted on discs floated upon solutions containing 1-2 μg/ml of metalaxyl. By contrast the development of an isolate showing resistance to metalaxyl sprays in the field was not affected by the presence of 100 μg/ml of this fungicide.

The technique was used during the summer in 1980 to survey potato crops in the S.W. of England for the presence of metalaxyl-resistant forms of *P. infestans*. Half the crops had received one or more sprays of a formulated mixture of metalaxyl and mancozeb ('Fubol') and half had been treated with other fungicides (not acylalanines) or were unsprayed. Metalaxyl alone had not been used. No metalaxyl-resistant forms were detected.

The technique is also proving useful in studies on the stability and relative fitness of metalaxyl-resistant forms of *P. infestans*. There are indications that the resistant forms are genetically stable, highly pathogenic and compete well with sensitive ones.

Variation for sensitivity to metalaxyl in Bremia lactucae (lettuce downy mildew)

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A method for testing *Bremia lactucae* isolates for sensitivity to metalaxyl *in vivo* is described. In a preliminary study it was found that metalaxyl applied to the roots of young lettuce seedlings at concentrations from 0.0001-0.1 μg/ml produced a range of responses from no activity to complete inhibition of sporulation. Using radioactive labelled metalaxyl, it was shown that the concentration of chemical in the cotyledons of test plants was similar to that initially applied to the roots. Two concentrations, 0.01 and 0.1 μg/ml, were subsequently used for screening isolates obtained from a laboratory collection and commercial lettuce crops. None of the isolates tested sporulated at the higher concentration over the period of the test; at the lower concentration there was some variation between isolates in the intensity of sporulation observed. An attempt to select metalaxyl insensitive mutants from spore populations irradiated with UV was unsuccessful.

No isolates of *B. lactucae* have been located which are sufficiently insensitive to metalaxyl to give cause for concern in relation to disease control.

Presence of some selective, genetically active fungitoxicant(s) in batches of formulated metalaxyl

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Preparations obtained from some but not all batches of Ridomil 25 WP by ethanolic extraction are fairly toxic to diploid strains of *Aspergillus nidulans* and highly active in increasing the frequency of mitotic segregation. This activity is not due to metalaxyl, several samples of which have been found to be only slightly toxic to the test organisms and genetically inactive. Column and thin layer chromatography have

been used to separate a genetically active material from metalaxyl. This material is highly toxic to *A. nidulans* (ED_{50} of approximately 0.2 μg/ml) and to several other higher fungi but has little effect on *Pythium* sp. at comparable concentrations. The main component of the genetically active fraction has been identified as methyl-D,L-N-(2,6-dimethylphenyl)-alaninate (CGA 67.891) which has been obtained in pure form and has been found without effect on mitotic segregation and only slightly toxic to higher fungi. Two minor components which have been detected but not identified appear to be present in very small amounts. This seems to indicate the presence of some fungitoxic compound(s) of extremely high potency.

Monilinia fructicola resistance to benomyl under field conditions

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In spring 1967, benomyl was tested for its efficacy in controlling brown rot of stone fruits caused by *Monilinia fructicola* and *M. laxa.* By 1972, the registration label permitted multiple applications during bloom and preharvest. Research evidence showed that in California a single spray applied before blossoms opened could protect stigma, anthers and petals of open blooms; an application three weeks before harvest protected fruits until harvest. Monitoring for benomyl-resistant *Monilinia* in 1973 failed to detect 1.0 μg/ml-benomyl resistance in California; yet where multiple sprays were applied exclusively on cherries from 1973-75 resistance was reported in the states of Michigan and New York (USA) in 1976; where benomyl had been used extensively after 1970, resistance was indicated in the 1974-75 season in Australia. Finally, in fall 1977, benomyl-resistant *M. fructicola* was detected in a few orchards in California, and then only at low levels of benomyl (0.5 to 4.0 μg/ml) as compared to as high as 800 μg/ml in Michigan and 50 μg/ml in New York.

Experimentation in orchards with populations of *M. fructicola* with low level benomyl resistance indicated that applications of alternate fungicides, such as captan, did not increase the number of resistant populations or the level of benomyl resistance, whereas the use of benomyl alone or in combination with captan increased the number of benomyl-resistant isolates and, furthermore, increased their levels of resistance. These resistant populations overwintered in affected mummies and blighted blossoms and twigs. Ascospores from apothecia found in orchards with benomyl-resistant isolates formed colonies similar to those produced by conidia from mummies and blighted blossoms. Blossom infections and colonization were influenced by the rate of fungal growth of specific isolates regardless of their resistance or sensitivity to benomyl. While on the fruit, inoculations with both resistant and sensitive isolates showed that sentitive isolates predominated in causing decay. Culturing benomyl-resistant isolates on a synthetic medium appeared to reduce their resistance to benomyl, which suggests that orchards not receiving benzimidazole

treatments may eventually develop a predominance of sensitive isolates. In recent studies with benomyl-resistant *M. laxa,* it was found that isolates often showed subnormal germ-tube growth, reduced sporulation, and had a diminished ability to induce twig cankers.

In orchards with benomyl-resistant *M. fructicola,* benomyl provided effective disease control if the level of resistance was 0.5 to 2.0 μg/ml, the resistant population was limited, and the disease pressure was minimal; under high disease pressure, effective disease control was not possible with benomyl alone or in combination with captan. Only alternative unrelated fungicides, such as triforine or dichlone, then provided effective disease control. Benomyl-resistant *M. laxa* isolates have been detected in apricot orchards but not in almond or prune orchards in California where benomyl has been limited largely to a single spray during bloom. Thus it seems reasonable that reducing the exposure period of *M. fructicola* or *M. laxa* to this chemical would reduce the chances of selecting resistant isolates. This could possibly be accomplished by reducing the number of benomyl applications per year to one and by using alternative unrelated fungicides in all supplemental applications.

Inhibitory effect of sodium bicarbonate against fungicide-resistant plant pathogens

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Sodium bicarbonate (SBC) was found to have an inhibitory effect against citrus common green mold and cucumber powdery mildew. However, the inhibitory effect of SBC did vary within replicated experiments. SBC with glycerine fatty acid added to strengthen adhesiveness, to avoid crystallization and to obtain uniform distribution on plant leaves, strongly inhibited several plant diseases. SBC had an inhibitory effect against fungicide-resistant and sensitive plant pathogens, viz. *Penicillium digitatum, P. italicum* and *Sphaerotheca fuliginea.* The nature of the protective effect of SBC on these diseases may be due to inhibition of conidial germination, conidial formation and their dispersal.

Genetic aspects of the benzimidazole resistance in a natural population of Venturia inaequalis

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Resistance against benzimidazole fungicides in *Venturia inaequalis,* in Germany first reported in 1974, was found primarily near Hamburg in orchards in which application of these fungicides has failed in apple scab control. The causal fungus was isolated from locations with various histories of fungicide use and examined for resistance to these fungicides. In laboratory experiments with different concentrations (0.1-1000 μg/ml a.i.) of four benzimidazoles, a total of seven reaction types (highly sensitive to highly resistant) of the organism could be differentiated.

Monoconidial isolates with different levels of sensitivity and resistance were crossed *in vitro* and progeny was isolated as random ascospore progenies or ordered tetrads. Segregation of benzimidazole resistance in ascospore progenies from crosses of sensitive and resistant strains of *Venturia inaequalis* demonstrated monogenic inheritance indicating a single gene mutation. Quantitative studies concerning the level of resistance confirm the monofactorial inheritance. Differences in the fungicide response of progeny suggest the presence of additional factors modifying the level of resistance to benzimidazoles. Testing of tetrad progeny indicated that there was no genetic linkage between the gene for benzimidazole resistance and the gene for sexual compatibility in *Venturia inaequalis*.

The use of physiological races of Venturia spp. for early detection of resistance to fungicides

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There is potential danger in selecting and producing a resistant mutant and releasing it into natural populations of plant pathogenic fungi. However, it is important to study the ability of such a mutant to survive under natural conditions as a plant pathogen. Such information on the fitness of resistant mutants and the influence of the natural environment on its spread and persistence would be invaluable in the management of current and new fungicides. In our research on development and epidemiology of fungicide-resistant plant pathogens, *Venturia pirina* and *V. inaequalis* have been used for early detection of resistance.

The commercial pear cultivars in Israel ('Spadona' and 'Gentile') are attacked by race II only of *V. pirina*. The introduction of benzimidazole fungicides improved scab control and since 1970 benomyl has been used extensively in pear orchards in Israel. In 1975 *V. pirina* (race II) was found to be resistant to benomyl, and during the following years resistant strains were noted in more than half of the pear orchards screened. Other races of *V. pirina* exist (I, II, IV, V) which do not infect the commercial cultivars and are restricted to their respective non-agricultural hosts. These races, which have never been exposed to fungicides, could be used for induction of resistance to fungicides, without threatening the commercial cultivars. Race V of *V. pirina* and its exclusive host, the wild pear *Pyrus syriaca,* was used to stimulate benomyl resistance in race V populations under the selection pressure of benomyl. Preliminary tests which included several million spores of race V from five single trees, did not reveal any benomyl resistance in the natural population before it was exposed to benomyl. Since 1979 several benomyl spray treatments have been applied to these single *P. syriaca* trees, but no resistance has been found. Similar tests were carried out also on the populations of races I and IV.

Apple scab is widespread in the commercial apple orchards in Israel. Benomyl has been used for the control of apple scab but it has not been used as extensively as for pear scab control. Screening for benomyl resistance since 1975 has not yet revealed benomyl resistance in *V. inaequalis* in Israel. The local cultivars, such as 'Hashabi', which has no commercial value, are heavily infected with scab and have never been

sprayed with fungicides. It was found that 'Hashabi' trees are naturally infected with a distinct strain of the scab pathogen (the 'Hashabi' strain) which is not able to infect the commercial apple cultivars. This means that the population of the 'Hashabi' strain has not been under the selection pressure of benomyl (and in fact no resistance was found). Moreover, *V. inaequalis* strains, which infect commercial apple cultivars, were found to be pathogenic to potted 'Hashabi' cuttings in greenhouse artificial inoculation tests. In the spring of 1981 single trees of 'Hashabi' naturally infected with the 'Hashabi' strain were sprayed with benomyl. No evidence of benomyl resistance has been obtained, although 10^7 spores were screened after exposure of the 'Hashabi' strain population to benomyl.

Benomyl resistance in apple scab; effect of inoculum concentration on competition between resistant and sensitive strains

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Resistance of the scab fungus *Venturia inaequalis* was first noticed in France in 1978. Competition between sensitive and resistant strains, isolated from a commercial apple orchard has been studied. It was found that a) the increase of the resistant part of a mixed population (one resistant strain versus one sensitive strain) showed a logit-linear relation (logit % resistant versus conidial generations), b) the slopes of the plots were independent of the initial ratio R/S (the total R + S was constant) and c) the slopes of the plots were a function of the total R + S inoculum. The less fit phenotype had a better chance to survive if the level of inoculum (or the distribution on the leaf surface) remained low during successive generations. Experiments as described cannot simulate the situation occurring in orchards but help to understand the mechanism of interaction between resistant and sensitive strains.

Competition between benomyl-resistant and benomyl-sensitive strains of Venturia inaequalis on apple seedlings treated with benomyl and captan

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The competitive ability of isolates of the apple scab pathogen resistant or sensitive to benomyl, was tested on apple seedlings. In the initial *in vitro* tests the differences in sensitivity to benomyl were determined according to the germination of spores on media amended with 0, 0.5 or 5 μg/ml benomyl. The sensitive isolate (GH) used had never been exposed to benzimidazole fungicides and was maintained in a greenhouse in Geneva, NY, by successive transfers to apple seedlings. The source of the resistant isolates was two apple orchards (Preston and Minns) in western New York, treated with benomyl but suffering from apple scab. An isolate from the Preston orchard was resistant to 0.5 μg/ml but sensitive to 5 μg/ml benomyl, whereas an isolate from

the Minns orchard was resistant to 50 μg/ml benomyl. The two resistant isolates did not differ in a pathogenicity test and caused typical scab lesions on seedlings treated with 150 μg/ml benomyl; the sensitive isolate did not infect the treated seedlings. Scab lesions from seedlings were used to prepare a mixed inoculum (5% resistant and 95% sensitive). The fitness of the two strains in the mixed populations was tested on untreated seedlings, and on seedlings treated with benomyl, captan, or a benomyl + captan combination. After four successive inoculations, the ratio of sensitive/resistant dit not change in the *Venturia inaequalis* mixed population from untreated or captan-treated plants, whereas in the benomyl or benomyl + captan treatments the *V. inaequalis* population became resistant. However, the rate of infection on plants treated with the combination was low, and the spore germination and the ability of the inoculum to infect apple seedlings was impaired.

Five years' model experiments on carbendazim resistance of the eyespot fungus under wheat field conditions

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In field populations of *Pseudocercosporella herpotrichoides* (Fron) Deighton, carbendazim-resistant types are present in the order of about $1 : 10^9$. In the absence of the chemical, there is no indication for lower or higher fitness of resistant strains in comparison to sensitive strains. Since spreading of the fungus in the field is extremely slow, it is possible to determine, how the relative frequency of carbendazim-resistant types changes successively in a wheat field with normal carbendazim application (trade name Derosal).

A 1-ha field was divided into two equal plots, one of which remained untreated during five years of continuous wheat cultivation. In the other plot, usually carbendazim (180 g a.i. per ha) was applied after shooting and at heading; for the last two years seed was also treated. Each year before harvest, samples of stubbles were taken from each plot and a representative number of isolates of *P. herpotrichoides* were made in the laboratory. After subsequent mass production of conidia, spore suspensions were poured onto agar medium amended with 3 μg/ml carbendazim. After two weeks, fungicide resistant colonies were counted and their frequency relative to the original number of conidia were taken as criterion to determine resistance of the population of the fungus in the field.

In the first year, one spore out of 2.4 billion was resistant in spore suspensions from the untreated plot. In the same year, the corresponding figure for the treated plot was $1 : 0.3 \times 10^9$, indicating an eightfold increase in frequency within a few weeks. However, after four other successive years, the relative amount of resistant conidia from the untreated plot was $1 : 0.18 \times 10^9$, and $1 : 0.10 \times 10^9$ in the treated one.

Although the linear increase in carbendazim-resistant populations and the differences between the two plots were statistically highly significant, the final figures still were in the order of 10^{-8}only. Conclusively then, for practitioners, there seems to

be no danger from carbendazim-resistance of the eyespot fungus, if the chemical is applied properly.

Comparable results and conclusions were obtained with *P. herpotrichoides* in rye after four years, and with *Septoria nodorum* in wheat during five successive years.

The effect of single and combination applications of benomyl and chlorothalonil on the level of benomyl tolerance in a natural population of Sclerotinia homoeocarpa and on the attendant development of turfgrass dollar spot

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Alternating or combination fungicide regimes have been suggested for managing the level of fungicide tolerance in fungal populations. This approach, however, was ineffective over one growing season in modifying the tolerance level in a natural population of *Sclerotinia homoeocarpa.* A creeping bentgrass golf course fairway with a high level of benomyl-tolerant components in the natural population of *S. homoeocarpa* was treated on a 2-week schedule with benomyl and chlorothalonil singly, in combination, and in alternation. Dollar spot severity was evaluated and the extant *S. homoeocarpa* population was sampled biweekly throughout June, July, and August, 1980. All recovered *S. homoeocarpa* isolates were tested *in vitro* for sensitivity to benomyl. Low rates of chlorothalonil provided excellent disease control. There was no disease control benefit from the combinations of benomyl/chlorothalonil over chlorothalonil alone. Of the 4052 recovered isolates of *S. homoeocarpa,* all but 25 (0.6%) were tolerant to benomyl *in vitro* ($ED_{50} > 1$ μg/ml). This high level of benomyl-tolerant components was stable across all treatments at all sampling times.

Benomyl-resistant and sensitive monoascosporic isolates of Monilinia fructicola

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From a peach orchard in California heavily infected with brown rot in the previous season, 80 monoascosporic isolates were obtained from apothecia of *Monilinia fructicola.* The isolates were found to differ in sensitivity to benomyl on media amended with 1 μg/ml of the fungicide. In the primary *in vitro* test 22 isolates originating from two apothecia were sensitive to 1 μg/ml, whereas the other 58 isolates originating from six other apothecia were resistant to 1 μg/ml benomyl. When the resistant cultures were tested on media with 1, 3, 5 or 10 μg/ml benomyl, seven were able to grow on 3 μg/ml and 16 on 5 μg/ml; no growth was observed during 21 days on 10 μg/ml. Some isolates differing in resistance levels were obtained from the same apothecium. The level of resistance remained stable even after ten successive transfers on benomyl-free media. The sensitive and resistant isolates were similar in

virulence, as evidenced by brown rot typical for *M. fructicola* on artificially infected cherries. However, the resistant isolates caused the same disease symptoms on cherries treated before inoculation with 300 μg/ml benomyl; this was not the case with sensitive isolates. No difference was found between the 1-, 3- or 5-μg/ml resistant isolates in their ability to infect cherry fruit treated with the high concentration of 1200 μg/ml of benomyl. The respective levels of resistance or sensitivity remained stable after re-isolations from the infected fruits. Brown rot did not develop on cherries treated with 50 μg/ml of CGA 64251 (1-((2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl) methyl)-1H-1,2,4-triazole) before inoculation, with either benomyl-sensitive or benomyl-resistant isolates.

Fungicide resistance in Turkey

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Vegetable crops, grown in greenhouses in the south and south-west coastal area of Turkey, suffer from several diseases.

In spite of an intensive spray programme with fungicides, growers increasingly complained about reduced effect of several chemicals. In view of this, pathogen isolates obtained from greenhouse crops were tested with respect to sensitivity *in vitro* against the fungicides concerned, namely carbendazim, thiram and mancozeb.

Carbendazim resistance was found in the following pathogens:

– *Botrytis cinerea* from cucumber, tomato, pepper and egg plant; six out of twelve isolates were able to grow on agar containing 1500 μg/ml carbendazim.

– *Fusarium oxysporum* f. sp. *cucumerinum* from cucumber; one out of ten isolates was able to grow on agar containing 500 μg/ml carbendazim.

– *Rhizoctonia solani* from tomato; one out of four isolates was able to grow on agar, containing 5 μg/ml carbendazim.

– *Sclerotinia sclerotiorum* from cucumber; three out of six isolates were able to grow on agar containing 5 μg/ml carbendazim.

– *Cladosporium* spp. from tomato, watermelon and cucumber; one isolate of *Cladosporium fulvum* and two isolates of other *Cladosporium* spp. were able to grow on agar containing 1000 μg/ml carbendazim.

Growth of non-resistant isolates of these pathogens is normally inhibited at 1 μg/ml of carbendazim in the agar medium.

Some reduced sensitivity to mancozeb and thiram was noticed in *Botrytis cinerea* from cucumber, tomato, pepper and egg-plant; five out of twelve isolates still showed a very limited growth at 5000 μg/ml of mancozeb in the agar medium, while the other isolates were completely inhibited at 1500 μg/ml. Similarly a slight reduction of sensitivity to mancozeb was found in isolates from *Cladosporium* spp. and to thiram in isolates from *Fusarium oxysporum* f. sp. *cucumerinum, S. sclerotiorum* and *Cladosporium* spp.

Resistance to fungicides in Aspergillus flavus

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Isolates of *Aspergillus flavus* Link. from the rhizosphere, rhizoplane, geocarposphere, spermosphere and kernel of groundnut peanut *(Arachis hypogaea* L) were tested for their sensitivity to eleven fungicides (PCNB, carbendazim, copper oxychloride, N-(ethylmercuri)-*p*-toluene sulphonanilide, captafol, zineb, mancozeb, captan, ferbam, thiram and thiophanate methyl). Amongst them isolate AF 50 was found to be resistant to all eleven fungicides. Other isolates showed a varying degree of resistance; isolate AF 35 was sensitive. The resistant and sensitive isolates were shown to be different in several physiological aspects.

On the mode of action of metalaxyl in Pythium splendens

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Growth inhibition of mycelial pellets of *Pythium splendens* by the acylalanine metalaxyl started 4 h after addition of 0.1 and 0.3 μg/ml of this toxicant. However, at these concentrations germ tube development in hyphal swellings of this organism was not prevented.

Using radiolabelled precursors inhibition of various biosynthetic processes was studied in mycelial pellets of *P. splendens* at growth inhibiting concentrations. RNA and DNA synthesis appeared to be strongly inhibited already ½ h after addition of the toxicant. Protein synthesis was not inhibited whereas lipid synthesis was almost unaffected at 0.1 μg/ml but strongly affected at 0.3 μg/ml of metalaxyl. At the latter concentration uptake of acetate as a precursor of lipid synthesis was also strongly inhibited.

Although the exact site of action of metalaxyl was not elucidated, it appears to interfere primarily with nucleic acid synthesis in *P. splendens* in a quite specific way.

Mechanism of action of metalaxyl in Phytophthora megasperma f. sp. medicaginis

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The effect of metalaxyl on RNA, DNA and protein synthesis has been studied in 20-h-old liquid cultures of *Phytophthora megasperma* f. sp. *medicaginis*. Cultures were initiated with encysted zoospores in a synthetic medium.

Almost immediately after addition of metalaxyl at 0.1 μg/ml the [^{3}H]uridine incorporation into RNA was inhibited for 70-80%, whereas the inhibition of [methyl-^{3}H]thymidine incorporation into DNA was lower and also more variable (0-40%). The incorporation of [^{14}C]phenylalanine into protein was hardly inhibited (10-15%).

The increase of RNA content of the cultures measured over a 6-h period was inhibited for 80% as has been determined with a colorimetric assay. Under the same experimental conditions no inhibitory effect of metalaxyl on the increase of the DNA content could be demonstrated.

Since metalaxyl neither inhibited the uptake nor the conversion of [^{3}H]uridine into [^{3}H]UTP it can be concluded that metalaxyl interferes with RNA synthesis. Whether DNA synthesis was also affected is not clear. A difference in pool size of dTTP between metalaxyl-treated and non-treated cultures might explain the observed inhibition of the incorporation of [methyl-^{3}H]thymidine into DNA.

The inhibition of RNA synthesis was concentration-dependent up to 0.1 μg/ml, and was at most 80%. At 1 and 10 μg/ml no further increase in inhibition occurred. It may indicate that the synthesis of only one type of RNA is inhibited.

Preliminary experiments with cell free extracts of the fungus revealed that metalaxyl does not directly affect the activity of RNA polymerase I and II.

From in vivo labelling studies and product analyses it appeared that metalaxyl differentially inhibits the synthesis of poly(A)+ RNA and poly(A)− RNA. The latter was significantly more inhibited than the former.

Additional research will be necessary to elucidate the site of interaction of metalaxyl within the complex process of RNA synthesis.

Mode of action of hydroxypyrimidine fungicides

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Hydroxypyrimidine fungicides act only against powdery mildews and this specificity is correlated with their effects on adenosine deaminase (ADA-ase). With few exceptions, hydroxypyrimidine analogues that are poor ADA-ase inhibitors are also poor mildewicides. ADA-ase is involved in purine metabolism in barley powdery mildew *(Erysiphe graminis* f.sp. *hordei)*, but not in two other barley diseases, leafscald *(Rhynchosporium secalis)* and brown rust *(Puccinia hordei)*. If present in other fungi ADA-ase is unaffected by hydroxypyrimidines. This unusual mildew enzyme may differ from fungal ADA-ase in other ways, for activity in mildewed barley is associated with a large protein (M.W. approx. 250 000), resembling that from mammals, and which is stable at low ionic strengths. In common with ADA-ases from other microbial sources, that from *Neurospora crassa* is unstable under these conditions. Since healthy plants generally lack ADA-ase, it not only offers an ideal target for fungicide attack, but might be a suitable enzyme to explore rational approaches to developing more potent fungicides.