TOPIC 3C

RESISTANCE TO PESTICIDES

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SYMPOSIUM PAPERS	3C–S1 to 3C–S4
RESEARCH REPORTS	3C–R1 to 3C–R25

3C—S1 RECENT PRACTICAL EXPERIENCES WITH FUNGICIDE RESISTANCE
T. Staub and D. Sozzi

3C—S2 INSECTICIDE RESISTANCE IN RICE PESTS, WITH SPECIAL EMPHASIS ON THE BROWN PLANTHOPPER *(Nilaparvata lugens stål)*
T. Nagata

3C—S3 SPREAD AND ACTION OF HERBICIDE TOLERANCES AND USES IN CROP BREEDING
J. Gressel

3C—S4 STRATEGIES FOR TESTING AND MANAGEMENT OF FUNGICIDES FOR CONTROL OF MONILINIA IN STONE FRUIT CROPS
Joseph M. Ogawa

RECENT PRACTICAL EXPERIENCES WITH FUNGICIDE RESISTANCE

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ABSTRACT

Over the past few years, resistance has led to the reduced effectiveness of dicarboximide, acylalanine and benzimidazole fungicides against certain important target fungi. In some instances resistance has developed contrary to risk predictions from laboratory and epidemiological studies. Monitoring of resistance in field populations can be of value to asses the resistance risk, to prevent crop damage, to help define and to check the effectiveness of anti-resistance strategies. The sensitivity test methods have to be defined carefully for each fungus/fungicide combination. In addition, to establish sound base-line data reference strains should be included in each test.

Anti-resistance strategies should prevent or delay build-up of resistance, since fungicides often have to be withdrawn from areas with high levels of resistance. Circumstantial and experimental evidence is presented for acylalanines that mixtures with multi-site fungicides delay the build-up of resistance. Better performance and enforceability of pre-pack mixture strategies are additional criteria in their favor. In high risk situations and when dealing with unstable resistance, alternating such mixtures with unrelated chemicals should also be considered. For design and implementation of anti-resistance strategies closer cooperation between manufacturers, distributors, academia, extension services and registration agencies is needed.

INTRODUCTION

In recent years fungicide resistance has increasingly become a practical problem and a threat to the effectiveness of certain newer highly active fungicides. The most prominent cases of field resistance have occurred against dicarboximides in Botrytis, against acylalanines in certain foliar oomycetes and against benzimidazoles in Fusarium and, most recently, in Pseudocercosporella on cereals. This prompted substantial efforts from academia and industry to improve our understanding of the basis of resistance and to search for ways to cope with it. Workshops in Wageningen, which brought together the world's experts on fungicide resistance in 1980 and 1981, served to both summarize and disseminate current knowledge in this new field (Dekker and Georgopoulos 1982). Under the auspices of GIFAP, industry organized a seminar on the topic in Brussels in 1981, and this led to the establishment of FRAC (Fungicide Resistance Action Committee). Its goal is to bring producers of related fungicides together and help define research and use strategies which prolong the useful life of fungicides at risk (Anonymous 1982).

This paper summarizes recent practical experiences and research with fungicide resistance that may contribute to a better understanding of factors that favor its development and of strategies to cope with it.

PREDICTION OF RESISTANCE RISK FROM LABORATORY STUDIES AND EARLY MONITORING RESULTS.

Prediction of field resistance with any degree of precision from laboratory studies and early monitoring has proved extremely difficult. In some cases resistance developed contrary to predictions. For instance, residual fungicides with broad spectra of activity are generally considered low risk compounds; and yet resistance to mercury and tin compounds have occurred and a recent report indicates Botrytis can develop field resistance to captan (Pepin & MacPherson 1982). Resistance to benzimidazoles had occurred in many plant pathogens, but was judged unlikely in Pseudocercosporella herpotrichoides on cereals from monitoring results and epidemiological considerations (Fehrmann et al 1982). Yet, cases of field resistance in eyespot have occurred in some countries (Fehrmann, personal communication). For dicarboximides, resistant mutants of B. cinerea could readily be produced in vitro; and soon resistant strains were found in the field, without however being correlated with reduced disease control (Beever & Byrde 1982). This appeared to be due to a reduced fitness of the resistant strains. Upon prolonged use of dicarboximides under high infection pressure, however, clearly reduced performance was in some cases associated with the resistant strains (Schüepp & Siegfried 1983). This demonstrated that even where resistant strains show reduced fitness, resistance problems occur if prolonged and exclusive use of one fungicide group under high disease pressure is permitted. Inhibitors of C14-demethylation in sterol biosynthesis(DMI)of fungi may be another group of fungicides for which, in spite of laboratory indications for low risk, intensive use may lead to problems in practice. This possibility is indicated by the appearance of resistant strains of Sphaerotheca fuliginea which reduced efficacy of DMI on cucurbits in the Middle East (Eli Lilly,personal communication). In cereal powdery mildew, shifts in sensitivity to DMI have also been reported, though on these crops correlation between poor control and reduced sensitivity has not been established (Fletcher & Wolfe 1981). A vital and still unresolved question for DMI fungicides is whether cross-resistance occurs between them and morpholines, which act at a neighbouring metabolic site in sterol biosynthesis. First studies with acylalanines indicated that in vitro resistance was not expressed in vivo; and selection experiments with various pathogens in vivo did not yield resistant strains (Bruin 1980, Staub et al 1979). It was the use of mutagens in combination with in vitro selection of P. megasperma that demonstrated that Peronosporales can develop in vivo resistance to acylalanines (Davidse 1981). The pessimistic indications from this study were nearly simultanously confirmed by the appearence of resistance problems in cucumber downy mildew in Israel (Katan & Bashi 1981) and later in other crops (Staub & Sozzi 1981). Lettuce downy mildew, on the other hand, which was thought to be especially resistance prone, has so far not developed resistance to acylalanines (Wynn & Crute 1981).

These examples show, that the resistance risk for a new fungicide group can at best be determined in a very general way by laboratory studies and theoretical considerations. For each new group, extrapolations from laboratory studies to field conditions can be subject to a new set of uncertainties. In practice, stepwise selection for resistance and fitness, yet undiscovered rare mutants, or hidden epidemiological constraints, may lead the development of resistance in unexpected directions.

MONITORING FOR RESISTANCE IN THE FIELD

Monitoring for resistance in the field can be used in the following situations:

- Early monitoring during the introduction of a new fungicide to assess the resistance risk and to establish base-line sensitivity data
- Evaluating strategies to minimize resistance risk (mixtures, alternation programs, limited number of applications)
- Determining persistance of resistance in absence of selection pressure (after withdrawal of fungicide)
- Analyzing product failures

Depending on the purpose of the monitoring and the fungus/fungicide combination, different techniques have to be used to determine sensitivity.

Simple, fast techniques, such as spore germination tests on agar, are ideal in many cases. Many spores can be analyzed in a short time allowing detection of very low resistance levels in otherwise sensitive populations. Many fungi do not sporulate readily however and radial growth of mycelial mass transfers on fungicide amended agar may be used to determine resistance levels. Many modern fungicides (eg. acylalanines) show no activity against spore germination *in vitro* and *in vivo*, and for some fungicides *in vitro* inhibition of mycelial growth shows little correlation to their *in vivo* biological activity (Staub *et al* 1979). *In vivo* methods on leaf discs, detached leaves or entire plants have been developed to monitor sensitivity to these fungicides. Disadvantages of *in vivo* methods are the limited number of individual isolates that can be tested; in addition, tests with mixtures of sensitive and resistant spores give only sensitive or resistant results, not the precise ratio.

When mixtures of sporangia from *Phytophthora infestans* sensitive and resistant to metalaxyl were inoculated on leaf discs floating on a lethal dose of that fungicide (250 sporangia/disc) a resistant reaction was found on all discs down to a ratio of 10% resistant sporangia. At a ratio of 1% resistant sporangia a third of the discs still showed a resistant reaction, while with 0.1% a resistant reaction was found only sporadically. Since 10 leaf discs were used in these tests levels below 0.1% could not be detected in a given sample. The method was accurate enough, however, to detect resistant *Plasmopara viticola* present in vineyards treated with a mixture of metalaxyl plus folpet and where no appearent breakdown of control had occurred (Staub & Sozzi 1981). It was possible to detect resistance levels as low as 0.01%, when several samples were bulked up and submitted to a preselection round on treated plants in the laboratory before sensitivity testing.

When analyzing product failures a major part of the target population may actually be resistant due to the selection pressure from an "at risk" compound. Severe damage in the field may already have occurred by the time resistance is confirmed in the laboratory, as shown in table 1.

TABLE 1

Mean time course of events in P. infestans related to the resistance against metalaxyl used alone

Day	1	First indication of a problem
	5	50% attack in actively sporulating foci (sample collected)
	10	Up to 100% attack in actively sporulating foci
	20	Resistance confirmed in the laboratory

For rapid analysis and correct interpretation of such situations factors other than the resistance detection level become critical. Previously established test methods and base-line sensitivity data of wild type populations are needed to differentiate shifts in sensitivity, especially when the resistance factors involved are small. To compare results from different tests or test locations and to relate them to the base-line data,known sensitive and,if available, resistant reference isolates should be included in every test as standard procedure.

Thus, field monitoring with all its inadequacies is a valuable tool to assess aspects of the development of resistance to fungicides. However, since detection is often possible only in late phases of the overall selection process, and close to product failures, the major efforts must be directed at strategies to prevent or delay the build-up of resistance and at implementing them early.

EXPERIENCE WITH DIFFERENT WAYS TO COPE WITH FUNGICIDE RESISTANCE

Control of established resistant populations

Once a fungicide group has lost its effectiveness in the field, the level of resistance is often so high that increased rates are ineffective and the fungicide has to be withdrawn from use in that area. Synergists as they are known for some cases of insecticide resistance do not commonly exist for fungicides. This may be due to differing biochemical mechanisms of resistance. Severe cases of fungicide resistance appear to be based on alterations at the target site, whereas insecticide resistance commonly results from increased metabolic detoxification (Plapp 1979). One exception is resistance in Pyricularia oryzae to organophosphorus fungicides, which is due to increased metabolism and can be reversed by appropriate synergists (Katagiri 1980). The search for fungicides with negatively correlated cross-resistance, though interesting from a theoretical viewpoint, holds little promise in practice, because it generally does not operate against all resistant mutants.

Development of new fungicides with novel mechanisms of action is not rapid enough to keep pace with the emergence of resistance. In fact, there appears to be a tendency within the chemical industry to concentrate research for new molecules on the few most active fungicide groups introduced in the last decade. This underlines the importance of use strategies that prevent or delay the build-up of resistance against these few fungicidal mechanisms involved.

Design of strategies to prevent or delay resistance build-up

To design anti-resistance strategies for a given fungicide/fungus combination the factors that influence the resistance build-up have to be considered. For this purpose it is necessary to distinguish between inherent factors, relating to fungicide chemistry and fungus biology, and management factors relating to fungicide usage within the context of crop management.

TABLE 2

Factors influencing the resistance risk

Inherent factors (fungus biology, fungicide chemistry)
- biochemical mode of action
- fitness of resistant strains
- reproduction rate of target fungus
- spore mobility
- duration of high disease pressure (climate)

Management factors (fungicide usage, crop management)
- duration of exposure (in generations)
- presence of other controlling factors (effective mixture partners, host resistance)
- size of target population, escape, overkill (protective vs. curative use)
- proportion of crop area treated

Inherent factors determine the basic resistance risk for a fungicide/fungus combination in a given area; they are largely fixed and beyond our control. The second group of factors is related to crop and disease management and includes use of resistant cultivars and cultural practices that reduce the disease pressure; they are variously under the control of farmers, officials, or manufacturers of a fungicide. The higher the basic risk from the inherent factors, the more stringently should fungicide usage be defined.

Management factors like reduction of exposure time obviously reduce the resistance risk. It is less clear how the size of the treated population,overkill, partial kill and escape influence the appearence of resistance. Partial kill and escape which are favored by mathematical models (Kable & Jeffery 1980) seem risky elements with explosive diseases like late blight. A farmer would be ill advised to let such diseases intentionally get well established. Experience with metalaxyl suggests, that treatments after a substantial level of disease has developed should be avoided since it was usually under such conditions that resistance emerged first in a given area (Staub & Sozzi 1981).

Comparative merits of fungicide mixtures and alternations

There is general agreement that specific-site fungicides should not be used exclusively where prolonged periods of high disease pressure occur. The two basic strategies to avoid the exclusive use of a resistance-prone fungicide are use of fungicide mixtures and alternation of fungicides with differing modes of action. Where the risk of stable resistance is high like with benzimidazoles and acylalanines, the following points favor use of prepack mixtures wherever an effective multi-site mixing partner is available:

- better disease control (synergism, secondary diseases)
- reduced damage potential from resistance
- better enforceability

Enforceability is very crucial. It is not satisfactory with tank-mix recommendations or with alternations of single products. Where the basic mixture is judged too risky to be used throughout the season, it can be alternated with an unrelated chemical. Although prevention of a misuse of attractive, specific fungicides will never be absolute, it is clearly better if only prepack mixtures are available to farmers. It is noteworthy and somewhat frustrating that some registration agencies do not accept prevention of fungicide resistance as sufficient grounds for registering a mixture. For unstable types of resistance as seen in Botrytis against dicarboximides, the element of fungicide alternation should be included in the strategy, since it allows the population to shift back to normal sensitivity.

Experimental evidence for delaying the build-up of resistance by mixtures

The concept that mixtures delay the appearance of resistance is largely based on rather crude theoretical models and circumstantial evidence from practical experience (Delp 1980, Kable & Jeffery 1980). We recently tried to validate the mixture concept in growth chamber experiments with populations of P. infestans. Late blight epidemics were simulated in three growth rooms with comparable conditions on four successive sets of potato plants. The initial inoculum (containing 5 resistant and 50,000 sensitive sporangia/ml) was sprayed on the first set of untreated plants and served to start the epidemics in each room. Development of the epidemics was followed over 60 d on the 3 subsequent sets of treated plants in each room. Treatments were applied before introduction of plants into the growth room and included metalaxyl (5 ppm), metalaxyl + mancozeb (4+32 ppm) or mancozeb alone (48 ppm), respectively. Rates used were in the same ratio as recommended for field use and gave similar control under the experimental conditions. The number of plants per treatment was 156 for metalaxyl and metalaxyl + mancozeb, 72 for mancozeb alone. In each growth room 3 untreated plants were included to simulate escape. Successive plant sets overlapped for at least two weeks to assure progression of epidemics. The epidemics were comparable in the different treatments with disease levels ranging from 70-100% foliar attack. At the end of the experiment, on the 4th plant set, disease control was 78, 82 and 92% for metalaxyl, metalaxyl + mancozeb and mancozeb, respectively. Resistance was monitored throughout the experiment with the leaf disc assay. At each sampling time one isolate was taken per three plants. No samples were taken from untreated escape plants.

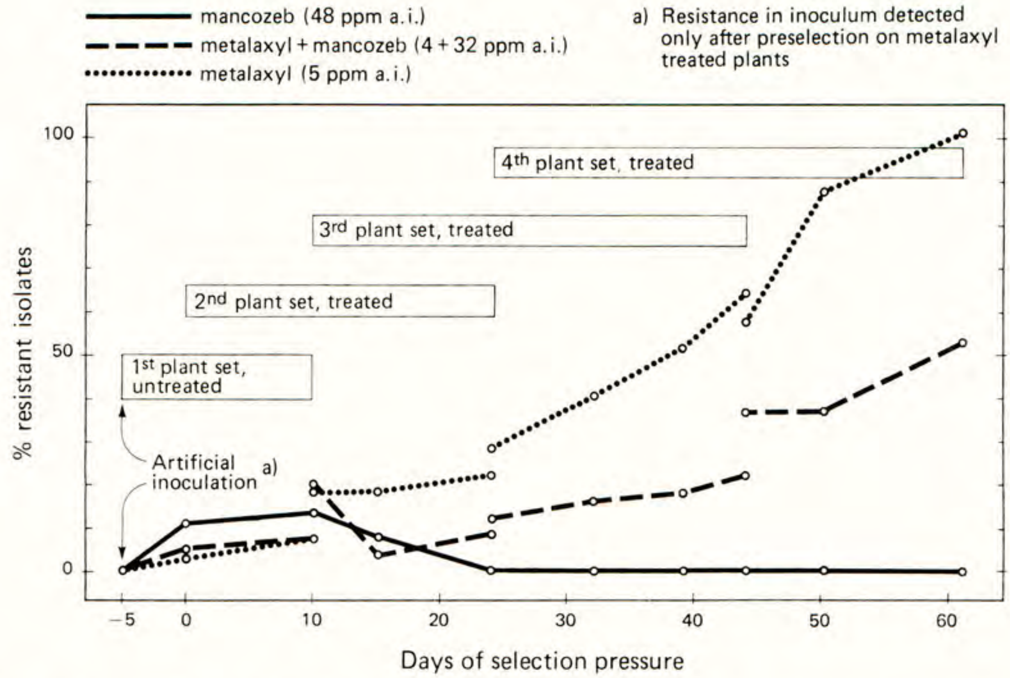

Fig. 1. Growth room simulation of resistance development from a mixed population of metalaxyl sensitive and resistant sporangia (10 000:1) of P. infestans on potato plants (cv.Bintje) under different spray schedules.(Resistance=sporulation at ≥10 ppm metalaxyl in leaf disc test)

A considerable delay in the build-up of resistance by the fungicide mixture could be shown (Fig.1). While metalaxyl alone resulted in 100% leaf discs showing resistance, the metalaxyl-mancozeb mixture schedule reached only 54% at the end of the experiment. Substantial disease control by metalaxyl alone at the end of the experiment illustrates, that the population was still partly sensitive although mass samples contained enough resistant spores to give a resistant reading. These data provide some support for the published mathematical models (Delp 1980, Kable & Jeffery 1980). However, these models have severe limitations especially where high disease control levels and low escape are involved. Reduction of resistance below detection level in the mancozeb treatment indicates a competitive disadvantage of the resistant isolate used.

Implementation of anti-resistance strategies

The implementation of anti-resistance strategies has proved to be a most critical point in many cases. The imagination of farmers seems unlimited when they are presented with new attractive fungicides. Reactions that have been observed range from introduction of highly susceptible cultivars because the fungicide is available to the use of mixtures in the soil against foliar pathogens, which prevents non-systemic mixture partners from becoming effective. Cooperation between producers of fungicides with cross-resistance is another weak link in the chain of elements that constitute an anti-resistance strategy. It is virtually useless for one distributor to exercise caution with a particular fungicide in order to reduce the resistance risk, if another one with a related fungicide does not follow suit. Reluctance of registration agencies to consider prepack mixtures is another obstacle which has to be faced in some countries.These examples serve to illustrate that enforceability of anti-resistance strategies has to be a key element in their evaluation. Independent of the strategies selected good coordination and cooperation between manufacturers and official extention and registration services is essential for their successful implementation.

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INSECTICIDE RESISTANCE IN RICE PESTS, WITH SPECIAL EMPHASIS ON THE BROWN PLANTHOPPER, (NILAPARVATA LUGENS STÅL)

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ABSTRACT

Insecticide resistance in Japan in two indigenous rice insects, Nephotettix cincticeps, Laodelphax striatellus and a migratory one, Nilaparvata lugens, is examined. N. cincticeps shows a high level of multiple resistance to organophosphates and carbamates. Sublethal doses of insecticides inhibit feeding of N. cincticeps and resistance to this action should be considered in relation to transmission of virus diseases. L. striatellus has also developed considerable resistance. However, though primarily indigenous, migration is likely to be a factor in the development of resistance in this insect. Resistance has been recently observed in immigrants of N. lugens which possibly results from changes in the origin of migratory insects. Knowledge of the geographical variations of this insect are important to determine the source of migration.

INTRODUCTION

Rice is cultivated over almost all Japan extending to 45°N and covering 2.4 m ha. It is subjected to a number of pest insects; rice stem borer, Chilo suppressaris, green rice leafhopper (GLH), Nephotettix cincticeps, brown planthopper (BPH), Nilaparvata lugens, white backed planthopper (WBPH), Sogatella furcifera, small brown planthopper (SBPH), Laodelphax striatellus, and rice leafroller, Gnaphalocrosis medinalis are all major rice pests throughout Japan. Leafminer, Agromyza oryzae, and rice leaf beetles, Oulema oryzea, occur only in the northern half of Japan. Rice water weevil, Lissorhoptrus oryzophilus, is a new rice pest which invaded from USA the central part of Japan in 1976, and its distribution is expanding. BPH, WBPH and the rice leafroller are characterized by long distance migration from outside Japan being incapable of overwintering there; all others are native indigenous species.

Japan was the first Asian country to introduce organic synthetic insecticides extensively for control of rice insects which has been achieved entirely with insecticides. Japan has the second largest annual production of insecticides in the world (300,000 t) and almost all is used on rice, which consequently has encouraged development of insecticide resistance in many species. Four rice pests are widely known to be resistant in Japan; GLH (organophosphates and carbamates), rice stem borer (organophosphates and BHC), SBPH (organophosphates and BHC) and rice leaf beetles (BHC).

Insecticide resistance in other Asian rice growing countries is still rare. This is mainly because insecticide use is still small, except perhaps in Taiwan and Korea. GLH (Ku et al 1976), BPH (Lin et al 1979) have been reported resistant in Taiwan as have SBPH and GLH in Korea (Choi et al 1975, Song et al 1976). Nephotettix virescens in Indonesia (Wickremasinghe & Elikawela 1982); and BPH in the Philippines (IRRI 1970, IRRI 1978). When

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compared with the high levels of resistance seen in GLH in Japan, most of these cases of resistance are low except perhaps for GLH in Taiwan and Korea.

In this article major cases of insecticide resistance in rice insects will be discussed with emphasis on the migratory planthoppers, especially in BPH.

INSECTICIDE RESISTANCE IN RICE LEAF- AND PLANTHOPPERS

Green rice leafhopper (GLH)

GLH insecticide resistance is the most serious among rice insects and detailed knowledge of biochemical aspects of this resistance has been compiled by Japanese workers. This pest is found in central and southern Japan and invades rice paddies from wild grass habitats after hibernation, and transmits a destructive virus disease, as well as causing damage by sucking plant juice. Insecticide application at an early stage of growth is recommended for virus control.

Malathion and parathion were the first insecticides used extensively against GLH. They were introduced in the beginning of the 1950s, and resistance developed in 1960-1961 in central Japan and expanded rapidly throughout central and southern Japan. Both organophosphates were then replaced with carbamate insecticides. A number of new carbamates were developed in the 1960s and were highly effective against organophosphate-resistant GLH (carbaryl, O-chlorophenyl methylcarbamate (CPMC), propoxur, 3,4-xylyl methylcarbamate (MPMC), Isoprocarb, M-tolyl methylcarbamate (MTMC), 3-sec-butylphenyl-N- methylcarbamate (BPMC), 3,5-xylyl methylcarbamate (XMC)). Exclusive use of carbamates against GLH also led to resistance.

In 1969 carbamate-resistant GLH was found in central Japan enlarging its distribution until the southern half of Japan was completely covered with resistant GLH by the middle of the 1970s. The degree of resistance was so high that topical LD50 values to some carbamates were about 100 times larger than those of a susceptible population. They also showed multiple resistance to malathion and some other organophosphates. However, some organophosphates such as diazinon or propaphos showed low levels of cross resistance and, as a result, were used to control carbamate-resistant GLH. In addition mixtures of organophosphate and carbamate, mixtures of fungicide and insecticide (s-Benzyl O,O-di-isopropyl phosphorothioate (IBP) + malathion, IBP + phenthoate), and a mixture of carbamate and its analogue (N-methylcarbamate + N-propylcarbamate) were all found to exhibit synergistic action against carbamate-resistant GLH. These mixtures are presently commercialized and used widely for the control of resistant GLH. However, GLH is developing resistance gradually even to these mixtures. Considerable effort is being directed towards developing new insecticides to overcome these GLH problems; to date results with some pyrethroids have been promising.

In Japan transplanting has been mechanized since 1969 which not only is this more labour-saving, but it allows more efficient insecticide application for GLH control. Disulfoton, propaphos or cartap granules are applied to the soil surface of each nursery box (28 x 58 cm) at the rate of 50-100 g/box immediately prior to transplanting. Control is good because granules embedded with rice seedlings around the root release active ingredient slowly and give long and persistent control. Disulfoton granules were used widely first. After 1978 reduced efficiency prompted replacement by propaphos or cartap granules.

In this application, insecticide is taken up by sucking insects such as

GLH only through oral route. Though resistance to systemic insecticides often is evaluated using topical application data that measure contact toxicity, other bioassay methods should be used to evaluate oral intake of systemic insecticides by sap-feeding insects. Feeding inhibitory action of insecticides at a sublethal dose suppresses virus transmission. Feeding inhibition can be measured readily by honey dew excretion when artificial diets containing insecticides are administered through an artificial membrane (Parafilm(R)). Cartap and propaphos showed conspicuous feeding inihibition at sublethal doses, but organophosphate- or carbamate resistance forms of GLH showed no cross resistance to cartap in relation to feeding inhibition (Nagata, unpublished) (Fig. 1). Therefore, resistance of GLH to systemic insecticides used for virus control should be judged not only by changes in lethal doses such as LD50 or LC50 values, but also by changes in doses needed to inhibit feeding.

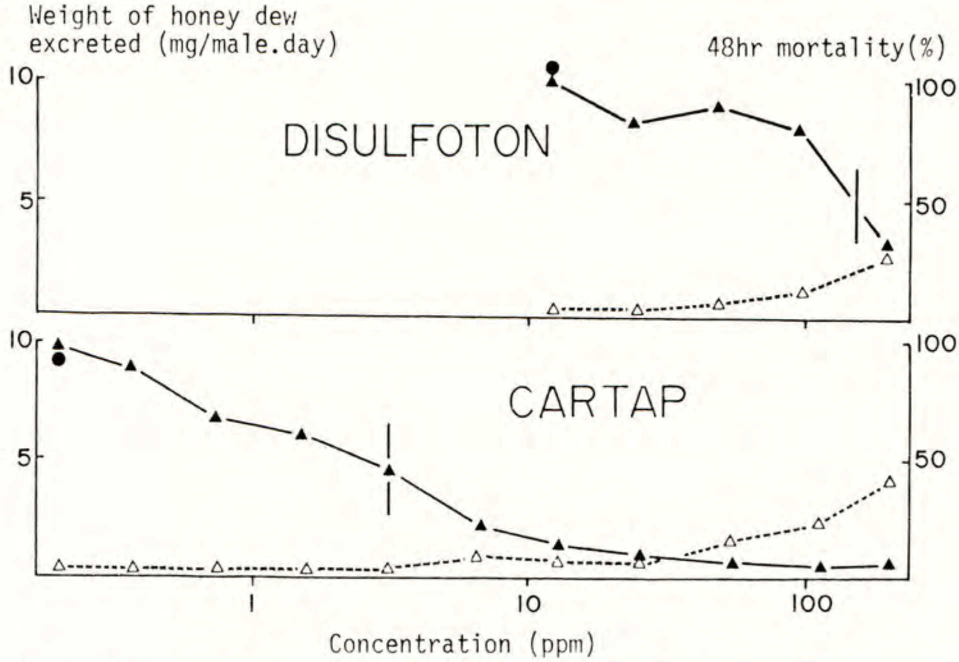

Fig. 1. Feeding inhibition by systemic insecticide administered to GLH (Resistant strain) through parafilm membrane. △ = 48 hrs mortality; ▲ = weight of honey dew; ● = weight of honey dew in control plot; | = 50% feeding inhibition dose.

Small brown planthopper (SBPH)

SBPH is a severe pest of rice and is the vector of stripe and black-streaked dwarf viruses. SBPH overwinters on wild grass at the fourth instar-nymph and adults emerge in spring moving first to wheat and then to rice paddies after transplanting. BHC and malathion have been predominantly used for control of SBPH since the 1950s, but resistance to both insecticides was reported in 1964 and they were replaced with fenthion, fenitrothion, disulfoton and vamidothion. A large decrease in the wheat crop, an alternative host, took place after 1969 due to Japanese government policy, and pest incidence was substantially reduced. However, since 1974, when the government began to enlarge the area planted to wheat again, SBPH has increased substantially in central and northern Japan.

Comparative data based on 1967 topical application assays showed that SBPH is developing a remarkable level of resistance to organophosphates and carbamates. A 1976 survey showed a 10-20 fold increase in LD50 to the organophosphates and data from several local populations collected at Kyushu in 1980 showed further increases in organophosphate resistance; 92-287 fold for malathion, 31-46 fold for fenitrothion, and 30-40 fold for diazinon. Carbamate resistance has also increased by: 9-21 fold for MTMC, 36-76 fold for carbaryl, and 23-26 fold for MIPC (Isoprocarb) (Nagata et al 1982) (Fig. 2).

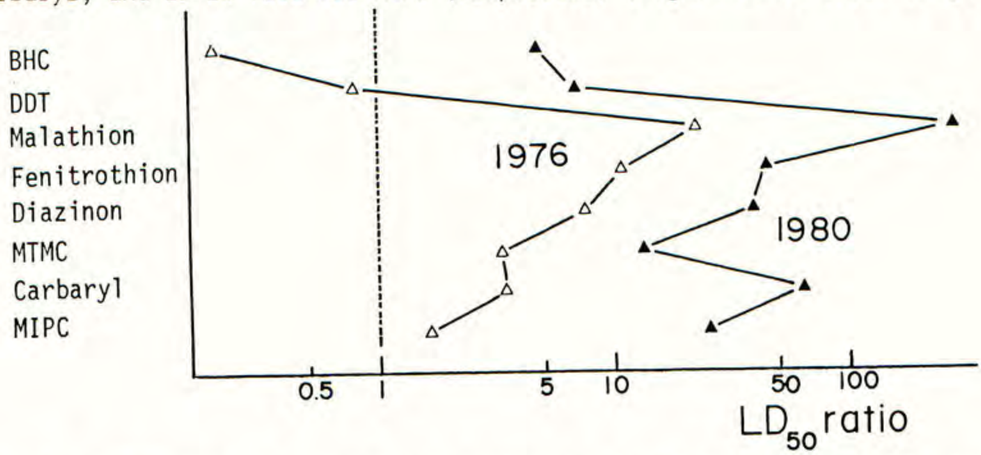

Fig. 2. Development of insecticide resistance in SBPH as expressed by resistance ratios on the basis of 1967 data. △ = 1976; ▲ = 1980

In 1980, comparative data from 7 local populations collected around Kyushu showed no significant difference in susceptibility to 8 insecticides tested. Furthermore, levels of susceptibility were similar to those of an SBPH population captured on a ship in the East China Sea at 31ºN, 120ºE 400 km from Shanghai, China. Further resistance data associated with migration of SBPH should be collected from sea-captured migrating SBPH and compared with land captured populations. Considerable numbers of SBPH are often collected on the East China Sea where the capture ratios were 3:1:0.4 for WBPH:BPH:SBPH respectively, between 1969-1979 (Kisimoto 1980).

SBPH is also an important rice pest in Korea, where insecticide resistance was reported in 1973 (Choi et al 1975) and again in 1974 (Song et al 1976). The level of resistance was almost the same as that in Japan. However, substantial local differences in resistance, especially for malathion, were observed. Choi et al (1976) reported an 8.4-fold difference among five locations, and Song et al (1976) reported 26.1-fold difference for malathion resistance.

Brown planthopper (BPH)

Insecticide resistance and migration

BPH is a rice pest of primary importance in southern Japan. It has also become a major pest of rice throughout much of Southeast Asia in the last ten years and its field control in tropical Asia relies heavily on use of resistant rice cultivars. However, insecticides are the sole control measure in Japan because commercial resistant cultivars have not been developed yet.

BHC and DDT were the first insecticides used for BPH control. BHC application predominated because it also controlled rice stem borer, WBPH and SBPH effectively, and cheaply. Although other rice insects developed remarkable resistance to BHC, BPH showed no substantial resistance after 20 years of continuous use (1949-1971) until its use was then banned because of the possibilities of environmental contamination. However, studies on reduced field efficacy of BHC against brown planthopper which occurred towards the end of the 1960s, suggested several interesting aspects of BPH insecticide resistance. One potent reason, perhaps, why BPH did not develop BHC resistance may be its high migratory activity. Long distance migration of WBPH and BPH from outside Japan was clarified in 1967 (Kisimoto 1971), although their population dynamics, especially in winter, had long been a subject of controversy amonst Japanese entomologists. Nagata and Moriya (1969a) observed loss of BHC resistance in BPH field populations which seemed associated with an influx of susceptible insects by long distance migration. Their research showed that in rice fields treated with BHC resistance to BHC fluctuated. BHC-resistant populations established following frequent insecticide applications in the autumn, die during winter, and resistance is lost by migration of susceptible BPH populations from less treated areas outside Japan during the next summer (Fig. 3).

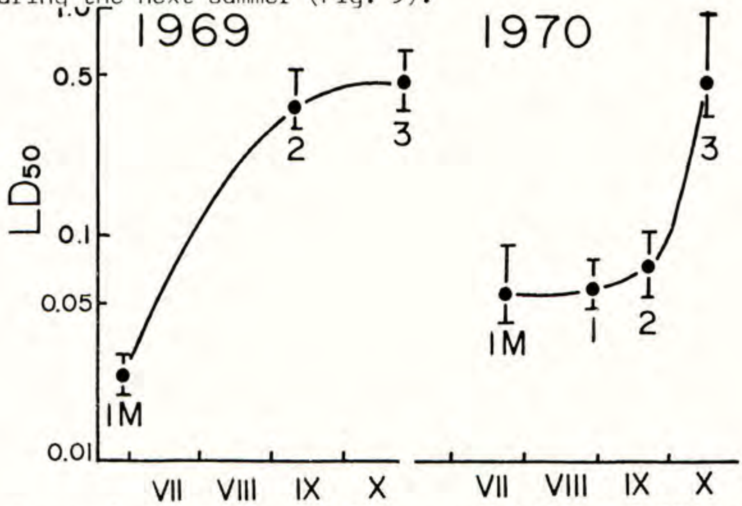

Fig. 3. Seasonal fluctuation of BHC-susceptibility of BPH in BHC treated rice paddy. LD50 = µg/female as indicated by topical LD50 ± 95% F.L.;IM = immigrant

Therefore insecticide resistance in Japan was considered to be primarily dependent on resistance levels of immigrants because effects of selection pressure by insecticides applied to paddies will not lead to an accumulation of overwintering resistant forms. It was recently found that immigrant BPH have developed resistance during the last ten years. When based on 1967 data, immigrant BPH populations collected at two monitoring sites on Kyushu island showed that LD50 to malathion and fenitrothion had increased significantly between 1967 and 1976, but no significant increase was observed for carbamates (Nagata et al 1979). By 1979 LD50 to organophosphates had increased further, and a 10-fold increase in carbamate resistance was recorded (Kilin et al 1981) (Fig. 4).

Cheng et al (1979) examined migration of BPH within China mainland. BPH can breed all year round on the southern half of Hainan island (25°N and southward), and its overwintering zone fluctuates between 21-25°N depending on the minimum temperature in winter. Northern migration begins in March and continues until August. There are five northward migration waves that finally reach 35°N and then three waves return migration southward begin. In China BPH has become serious since the 1970s and insecticides are important in BPH control because resistant rice varieties are not planted widely yet. In addition, migration of BPH from neighbouring tropical areas, e.g. Philippines, Vietnam, etc. to China, though not referred to in these Chinese reports, is highly probable. BPH populations flowing into China mix with BPH overwintering there and then multiply. A portion of the mixed poplation is likely to

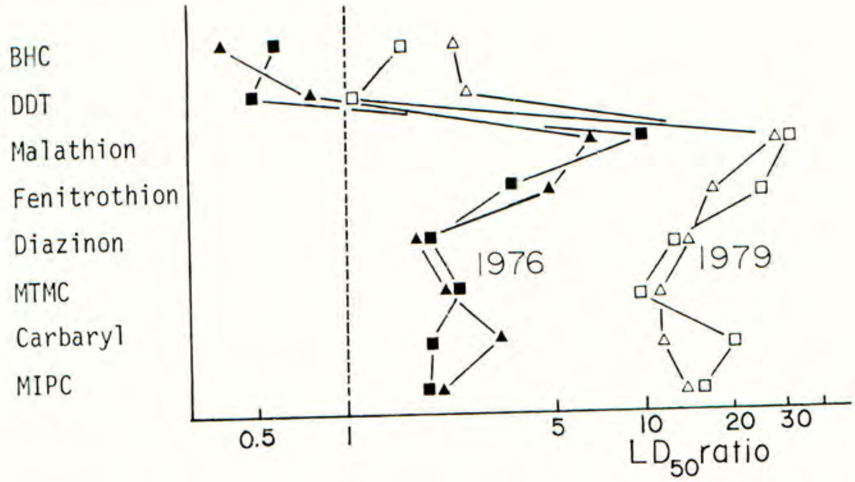

Fig. 4. Development of insecticide resistance in immigration BPH as expressed by resistance ratios on the basis of 1967 data.▲ = 1976 Kagoshima prefecture; ■ = 1976 Nagasaki pref.; Δ = 1979 Kagoshima pref; □ = 1979 Nagasaki pref.

be conveyed to Japan as part of the northward migration.

Therefore, resistance levels of the two populations (tropical population and the population overwintering in China) and the extent to which they mix will determine the composition and resistance levels to insecticides of immigrant populations reaching Japan. Regular monitoring of resistance levels of these BPH populations would be useful for predicting resistance levels of BPH invading Japan. Some toxicological data on BPH insecticide susceptibility in the tropics reported from Thailand (Nagata & Masuda 1980), the Philippines (IRRI 1979, Nagata & Masuda 1980) and Indonesia (Kilin, pers. com.) indicates BPH in these areas are generally more susceptible than in Japan. Although diazinon resistance and carbofuran resistance have been reported from the Philippines, both were confined to experimental fields in IRRI (IRRI 1970; IRRI 1978). BPH recently obtained from three locations in China (Shanghai, Hangchow, Kwangchow) showed identical patterns of insecticide susceptibility with those in Japan (Nagata, unpublished), and BPH with similar resistance levels were also collected from Taiwan in 1977 (Nagata & Masuda 1980). In Taiwan, however, BPH resistant to MTMC and MIPC has been reported (Lin et al 1979), and a 6.9-fold difference in LC50 to MTMC occurred amongst samples from 12 survey sites in Taiwan.

Laboratory selections have been conducted by many workers to predict future resistance levels of BPH. Selection with organophosphates or BHC readily produced resistant populations (Nagata & Moriya 1969b), but resistance development was generally very slow when selected with carbamates (Chung & Choi 1981, Hosoda, pers. com.). Conflicting results were obtained in Taiwan, however, where Chung et al (1982) obtained a 34-fold resistance over 16 generations of selection with MIPC. They also observed cross resistance between carbamates and some pyrethroids with the exception of fenvalerate.

White backed planthopper (WBPH), which migrate to Japan in greater numbers than BPH, is generally the most susceptible to insecticides of all planthoppers. This insect has not developed significant resistance. In 1980 a slight increase in LD50 was recorded, however, it was less than 5-fold when compared with 1967 baseline data (Nagata et al 1982).

Geographical variation of rice planthoppers

In the studies of insecticide resistance of migratory insects, it is essential to determine definite migration ranges for each insect. Detail of BPH migration, especially in the tropics, is not generally available. Characteristics of geographical BPH variation may help determine the migration range. Distribution of BPH biotypes with different virulence to resistant rice varieties has been reported from Sri Lanka (Fernand 1975) and northern India (Pathak & Verma 1980). BPH collected from 35 regions of China were determined to be biotype 1 (Wu et al 1981). By hybridization experiments and acoustic studies of mating signals Claridge (1981) concluded that BPH populations obtained from Australia and the Solomon islands were geographically distinct from the Philippine population. BPH and WBPH populations collected from the Philippines and Thailand retained significantly more brachypterous forms when compared with a Japanese population reared on rice seedlings (cv Japonica (Nagata & Masuda 1980) (Fig. 5). Tropical populations were generally more susceptible to insecticides, especially to DDT. BPH collected from Indonesia also gave a high proportion of brachypterous forms on laboratory rearing (Nagata, unpublished).

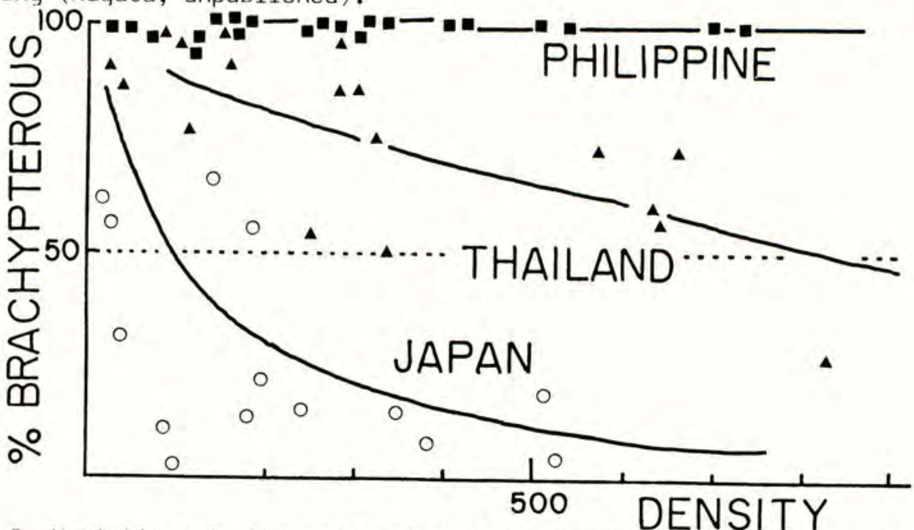

Fig. 5. Variations in the proportion of brachypterous females appearing on laboratory rearing. ■ = Philippines; ▲ = Thailand; ○ = Japan; Density = Total number of emerged adults/rearing pot

BPH immigrants invading Japan in the several waves of migration extending over a month often show divergent properties, which may indicate they are of different origins. For instance, variation in insecticide susceptibility and aliesterase activity were found between two migration waves (Hama, unpublished). Also ratios of brachypterous forms differed significantly between populations sampled from different migration waves when transferred to laboratory rearing (Nagata, unpublished). The significance of this genetic differentiation in wing-morph determination is not fully understood, but it will perhaps be elucidated in the forthcoming studies on BPH migration.

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SPREAD AND ACTION OF HERBICIDE TOLERANCES AND USES IN CROP BREEDING

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ABSTRACT

Localized cases of herbicide resistance and tolerance have appeared worldwide, almost exclusively to the s-triazines. Species have evolved s-triazine tolerance by increasing the rates of herbicide detoxification and to resistance by not binding herbicides to the chloroplast target. Selection of resistant crop strains by novel techniques has been confounded by lower progeny fitness.

INTRODUCTION: THE SPREAD

The first incidences of triazine resistant (Fig. 1) weeds were reported, in the mid 1970s. Shortly thereafter the problem was discounted as "not being serious, nor does it appear to be a major threat in the future" (Parochetti 1975). If that had turned out completely to be the case there would have been no justification for a recent book on the subject (LeBaron & Gressel 1982) nor for the following discussion. Among some, "there is a prevailing view that the problem of the development of resistance to herbicides mainly involves the replacement of sensitive species that originally were dominant.. with other species that never were sensitive" (Day 1983). Such ideas about changing distribution patterns have never been based on good numerical ecology of agricultural systems. In addition, in field agriculture it is rare that only one parameter is changed at a given time, making such "prevailing views" highly objective. There is one notable exception where there was an excellent data base before herbicide usage and good large scale measurements after; and the conclusions are ambiguous. Haas & Streibig (1982) directly correlated large scale changes in weed distribution with the usage of phenoxy herbicides in Danish small-grain fields. They also correlated the same new patterns of weeds with increased area drained, increased nitrogen fertilization, and the introduction of higher yielding varieties. These other factors were clearly as important herbicides. Weed species with an affinity to soggy soils disappeared as did those that did not respond to greater soil fertility. Throughout the remainder of this chapter, only cases where newly resistant biotypes appeared will be discussed. A theoretical, mathematical analysis had predicted that incidences of resistance to highly persistent herbicides used in monoherbicide-monocrop culture would increase exponentially over the years (Gressel & Segel 1982). Since the first reports in the mid 1970s, cases of triazine resistant populations have been reported in 37 species of 24 genera that were previously controlled (LeBaron pers. comm. 1983). Often the same species has become resistant in scores of locations. Except in Hungary where 75% of the maize growing area was rapidly covered by s-triazine resistant *Amaranthus retroflexus*, the incidences have been isolated to small areas and could be controlled by the use of alternative herbicides. The history of the spread of triazine resistance is described in detail (Bandeen *et al* 1982, Gressel *et al* 1982). Dicot weeds were the first to evolve triazine resistance. The majority of the newer reports are about triazine resistant grasses. Triazine resistant *Poa annua* has become problematic in Belgium (R. Bulcka, pers. comm. 1983) and Holland (van Dord 1982). The road authority in Israel has been spraying roadsides with triazines for 15 years and first *Brachypodium distachyon* (Gressel *et al* 1983) and then 6 other grass biotypes; *Phalaris paradoxa, Alopecurus utriculatus, A.myosuroides Lophochloa phleoides, Lolium rigidum, Polypogon monspeliensis* were found.

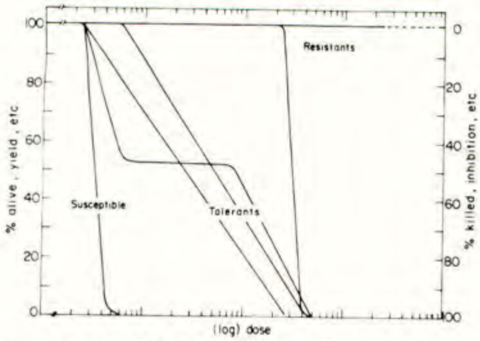

Fig. 1. Resistance is defined as being able to withstand an agricultural dose of the herbicide giving a normal yield. When a weed is only partially affected, it is tolerant. Individuals and populations can be called tolerant and resistant.

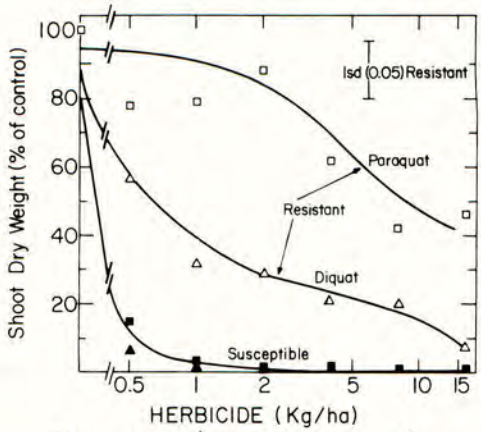

Fig. 2. The resistance of recently appeared biotype of *Erigeron philadelphicus* to paraquat and diquat 10 days after application (Redrawn from Watanabe *et al* 1982).

that were triazine resistant (Drs. A. Nir and B. Rubin pers. comms. 1983).Dicots resistant to triazines were first found in Ontario in 1974. Since 1980, *Panicum capillare, Echinochloa crusgalli* and *Setaria glauca* (all monocots) resistant biotypes have been found (Alex & McLaren pers. comm. 1983). Most cases of resistance have been to the s-triazine herbicides, but resistant biotypes have appeared to other, less widely used herbicides. *Eleusine indica* populations resistant to trifluralin have spread to at least 7 counties in South Carolina (LeBaron pers. comm. 1983). *Poa annua* resistant to paraquat has been reported and two species of *Conyza=Erigeron* have also evolved paraquat resistance; *C. linifolia* in Egypt and *E. philadelphicus* (Fig. 2) in Japan. *Lolium rigidum* resistant to diclofop-methyl has been found in Australia (Table 1)

Are some species genetically more adaptable, allowing them to evolve resistance more rapidly than others? Is it chance that *Lolium* biotypes resistant to diclofop and tolerant to paraquat and dalapon, have evolved in separate areas of the globe? Similarly; *Conyza=Erigeron* and *Poa annua* resistant to triazines and paraquat have been reported (cf. Gressel *et al* 1982). These cases of seemingly greater "adaptability" evolved in different areas. A population resistant to one of the herbicide groups is susceptible to the others. The "super-resistant" strain has not yet evolved; cross resistances have not occurred in weeds as they have in insects.

There is one commonality to all cases of resistance that have occurred to date. They have all evolved in situations of crop mono-culture where a single herbicide was used annually. Resistance to the predominantly maize herbicide atrazine did not occur first in the U.S. corn belt. Most farmers in the "cornbelt" practice crop rotation and use atrazine together with alachlor in maize. The first cases of triazine resistance in the cornbelt were not in maize; the railroads were using sterilant levels of simazine along their right-of-ways and inadvertantly selected for triazine resistant *Kochia scoparia* (cf. Bandeen *et al* 1982). The effects of passing trains blowing the seeds into fields has to be evaluated. There are now preliminary reports of triazine resistant *Amaranthus* in the cornbelt (H. LeBaron pers. comm. 1983). As this weed is alachlor sensitive, it is unlikely that those farmers used herbicide mixtures. Theoretical models have been used to suggest agronomic practices to delay (not prevent forever) the appearance of herbicide

TABLE 1 - Diclofop-methyl resistant *Lolium rigidum* from Austalia

Rate (a.i)	Wild type	Resistant biotype
	(% remaining alive)	
375 g/ha (normal rate)	20	100
1.5 kg/ha	0	90

Source: R. Knight, 1982

resistances (cf. Gressel & Segel 1982). One key factor governing the rate at which evolution to resistance will appear is the persistence of the herbicide; the greater the persistence, the quicker resistance will appear. It was thus surprising that resistance to paraquat appeared; this is one of the least biologically persistent herbicides available. The biological persistence of paraquat was compensated for by the persistence of the farmers; in Egyptian orange groves it was sprayed at almost monthly intervals to get to the resistant *Conyza* (cf. Gressel *et al* 1982) and in Japan 2-3x annually for 8-11 years in mulberries to select for the resistant *Erigeron* (Fig. 2). Paraquat was not rotated with other herbicides.

THE APPEARANCE OF RESISTANCE VS. TOLERANCE

Resistance

The models describing the appearance of resistance suggest that the enrichment for a rare one or two gene mutant (resistant) individual in a huge population is an exponential process. Thus it will take years to get from the 10^{-5} to 10^{-12} expected initial frequency of resistant individuals to a noticeable 10%. Theory was not backed by data until Nosticzius *et al* (1979) counted weeds in maize fields with a data base beginning before the usage of atrazine. Their data clearly show the jump in resistance expected when the enrichment is plotted on a linear scale (Fig. 3). Thus, the field observation that "we have not seen any sign of resistance appearing" is meaningless. We will not see resistance until its fully upon us. The appearance of triazine resistance in the same species in different locations is probably due to concurrent evolution; not to spread. Different "chemotypes" of *Chenopodium album*

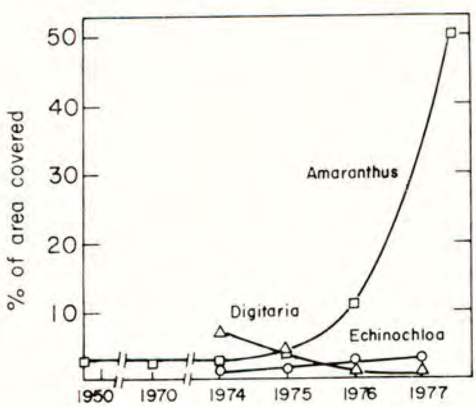

Fig. 3. Changes in weed populations in a monoculture maize field in Hungary treated annually with atrazine since 1970. (Data were plotted by Gressel & Segel, 1982, from Table 1 in Nosticzius *et al* 1979; (Reproduced with permission).

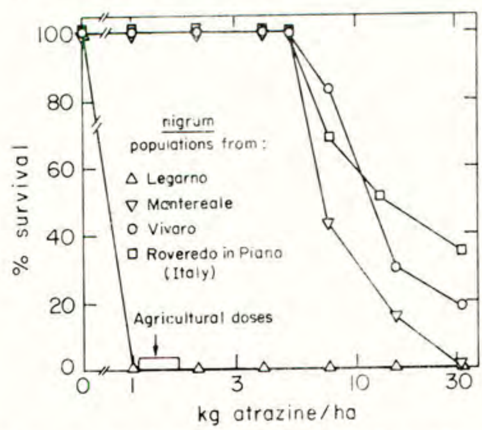

Fig. 4. Variable response of s-atrazine resistant *Solanum nigrum* accessions. Seeds of the resistant biotypes were gathered in northern Italy and assayed in pot tests. Plotted by Gressel (1984) from data of Zanin *et al* (1981) (Reproduced with permission).

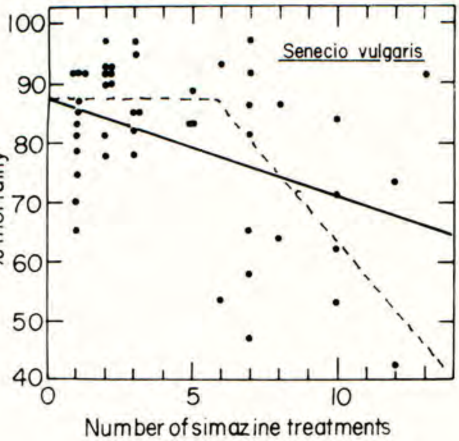

Fig. 5. Increased *Senecio vulgaris* tolerance to simazine as a result of repeated treatments. *Senecio* seeds were collected at 46 locations in England where the previous treatment history was known and then treated with 0.7 kg/ha simazine under standardized conditions, yielding the results in this figure. The variance due to the regression (solid line) was highly significant ($p<0.01$. The dashed line fits the explanation in the text. Plotted by Gressel & Segel (1982) from data in Holliday & Putwain (1980). (Reproduced by permission)

are found in the same field and triazine resistance appeared in different chemotypes in different locales (Gasquez & Compoint 1981). Separate evolution also seems evident from the slightly different dose response curves obtained from different *Solanum nigrum* accessions from different locations in Northern Italy (Fig. 4).

Tolerance

The increase in tolerance is far more insidious than resistance; more cases are documented (LeBaron & Gressel 1982). There are probably many genes involved, each having an additive effect (cf. Holliday & Putwain 1980, Faulkner 1982). There will not be the exponential jump from a herbicide-susceptible to a tolerant population as seen with resistance (Fig. 3). Instead, there should be a short delay of a few years until many individuals with partial tolerance are selected. As these partially tolerant individuals become predominant, their chances of interbreeding will increase. The continual selection pressure by the herbicide should bring about a further gradual increase in tolerance when the various genes conferring tolerance begin to interact. Such a gradual enrichment, after a short lag, was found in *Senecio vulgaris* populations with various simazine treatment histories (Holliday & Putwain 1980). There was a sporadic but continual increase in simazine tolerance after the fifth year of treatment (Fig. 5). The tolerance was statistically significant but the variability was great and was partially due to the use of other herbicides. Similar results have recently been found with *Avena fatua* (Table 2). Tolerance may best express itself agronomically as a need to increase the rate of herbicide application every few years to control weeds that were previously controlled. Stepwise increases in tolerance have been found with insecticides (Devonshire & Sawicki, 1979) and with cancer cells tolerant to anticancer drugs. In these cases, the "pesticide" is catabolized in the cells. Rare duplications of the genes for these enzymes are enriched for, with concomitant increases in enzyme level. In these cases and others previously described, the level of tolerance can continuously increase until the plants are truly resistant to field rates of pesticide.

Concurrent Evolution Towards Tolerance and Resistance

There is beginning to be some evidence that the same species can evolve both tolerance and resistance, as governed by different genes, in different locales. These include tolerant species such as *Echinochloa crus-galli* (cf. Gressel *et al* 1982) and *Senecio vulgaris* (Fig. 5) which have also evolved resistance at the plastid level (see following section) in other locales. There may have been evolution to both resistance and tolerance in *Brachypodium distachyon*. The plastids of the resistant biotype of this species have the

TABLE 2: Tolerance of *Avena fatua* populations to 1.2 kg/ha triallate

Previous treatment	%surviving to first leaf	treated÷control
Expt. A.		
None (control)	15	
4 y triallate	22	1.4
Expt. B		
none (control	8	
21 y diallate or triallate	33	4.1

Source: Condensed from Tables 3 and 4 in Jana & Naylor (1982)

immediate resistance response typical of other triazine resistant weeds (Gressel *et al* 1983). The resistant plants also degraded atrazine more rapidly than susceptible ones (Fig. 6). Total resistance may have appeared when the enrichment for tolerance had not yet arrived at a point where the plants are able to withstand high herbicide doses. If the tolerance is inherited in the nucleus and resistance in the plastome, we should be able to separate them in the offspring of the reciprocal crosses, crosses that are presently in progress in my laboratory.

MECHANISMS OF HERBICIDE RESISTANCE

The biochemical and physiological mechanisms controlling tolerance and resistance can provide considerable information about inheritance and then the modes of evolution leading towards the appearance of the resistant weed.

Triazine Tolerance and Resistance

Maize is the major crop in which s-triazine is used as a biochemically selective herbicide. The resistance in maize is due to a nuclearly inherited specific glutathione-S-transferase which conjugates with chloro-s-triazines. Mosttriazine resistant weeds do not have the same mechanism as maize. Unlike maize, their chloroplasts are totally resistant to triazines, which interfere with photosystem II of photosynthesis (cf. Radosevich & Holt 1982, Arntzen *et al* 1982). Triazines do not bind to the plastids of resistant biotypes (cf. Arntzen *et al.*, 1982),which protects them. This trait is maternally inherited (Souza-Machado 1982), presumably on the plastid genome. The agronomical implication of maternal inheritance is that the trait cannot be spread by pollen, only be seed. Duesing and Yuc (1983) have estimated that the frequency of triazine resistant individuals is $<10^{-10}$. They characterized a nuclear gene in a triazine resistant *Solanum nigrum* accession that increases the frequency of chloroplast mutations about a thousand fold. Such a plastome-mutator gene may be a transient step in the appearance of resistance that is quickly bred out of the populations. This property has not been seen in other triazine resistant biotype accessions.

There are slight biochemical differences in the triazine binding properties of the different biotypes; the ratio between binding constants of resistant to susceptible varies among species (cf. Arntzen *et al* 1982). There are also slight differences in the dose response curves of different resistant biotypes of the same species (Fig. 4). These may result from different amino-acid substitutions at various points in the triazine binding protein in photosystem II. The lesion responsible for non-binding of the herbicide probably causes the increase of the half-time of fluorescence decay (cf. Arntzen *et al* 1982) and the decrease in photosystem II efficiency in resistant plastids (Radosevich & Holt 1982). This could translate into the reduced fitness of these biotypes (see below). Many laboratories are trying to isolate, clone and sequence the gene for the triazine binding protein. Triazine resistant weeds may be more responsive to other herbicides. Some of the herbicides inhibiting photosystem II are clearly more toxic to the resistant than the

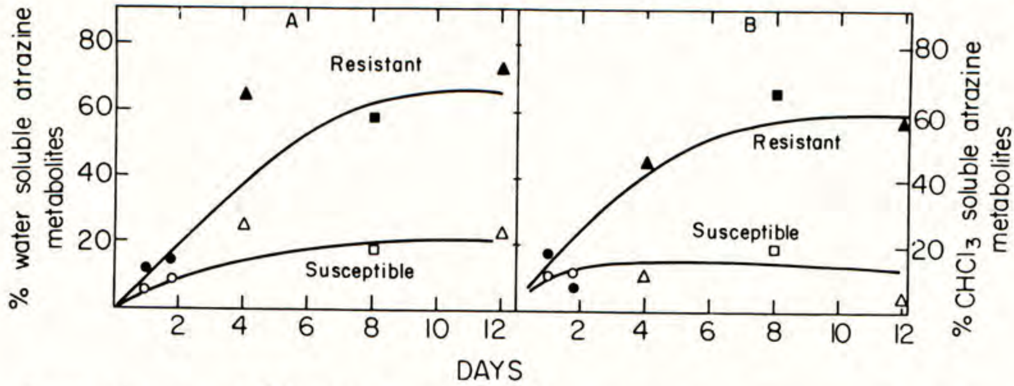

Fig. 6. Metabolism of ^{14}C-(ring)-atrazine by resistant and susceptible *Brachypodium distachyon* biotypes. A. To water soluble metabolites; the data are presented as percent of total that partitions into the aqueous phase. Water soluble metabolites are all inactive. B. To chloroform soluble components. The percent of radioactivity on TLC plates not in parent atrazine. Each symbol shape represents a separate experiment. (Modified from Gressel *et al.* 1983).

"wild type" plastids (Arntzen *et al* 1982). Diuron resistant (triazine tolerant) *Euglena* was also much more susceptible to EPTC (Laval-Martin *et al* 1983). These greater susceptibilities have not been checked, to my knowledge, under field conditions.

Paraquat

Paraquat interacts mainly with photosystem I, and its photoactivated form interacts with oxygen, forming highly reactive oxygen species. These in turn wreck havoc upon membranes and the sprayed leaves die within hours. Paraquat interacts with photosystem I in both tolerant and susceptible *Lolium*; resistance is not due to a lack of interaction with the target site. Resistance and tolerance have been correlated with a 200% and 50% increase in superoxide dismutase levels in *Conyza* and *Lolium*, respectively (cf. Harvey & Harper 1982). This enzyme is probably limiting for detoxifying the active oxygen; its product is toxic peroxide. The plastids must therefore have a highly active peroxide removal system. A coupled reaction through ascorbate, glutathione and NADPH has been proposed to remove the H_2O_2, as plastids do not contain peroxidases (Dodge 1983).

SELECTION FOR TRIAZINE RESISTANT CROPS

There is not always a good match between a crop, the weeds competing with it, and herbicides that can be cost-effectively used. In areas where maize has not been the major crop, triazines, especially atrazine would be very economical, if only they would not kill the crops. Only limited use has been made of breeding to obtain tolerant or resistant varieties of crops (Faulkner 1982). Triazine resistance has been transferred from the weed *Brassica campestris* to the *Brassica napa* crops; rape-seed and rutabaga by crossing and continual back crossing (Souza-Machado 1982). Cell culture selection systems allow the rapid selection for resistance with large populations in small volumes with short generation times. Their main drawback is the number of crops that can be regenerated. Various cell culture techniques have been used to obtain tobacco plants (separately) resistant to picloram, bentazon and phenmedipham (cf. Meredith and Carlson 1982).

Protoplast fusion techniques are being used in our laboratory to transfer the cytoplasmically inherited triazine resistance that has also appeared in many weeds that do not interbreed with crops, as in *Brassica* (see Section 5B of these proceedings).

The Fitness Enigma

More often than not, selected individuals are less "fit" or less competitive than the wild type. Unfortunately, no field data have been published for any of the herbicide resistant tobacco strains derived through cell cultures. Carlson (pers. comm. 1983) reported that his phenmedipham resistant tobacco performed very poorly in the field. The fitness problem is less "mystical" with the maternally inherited triazine resistance. Photosystem II electron transport was severely inhibited in each case looked at biochemically (Arntzen *et al* 1982; Radosevich & Holt 1982). The effect was less when CO_2 fixation was measured. In nature CO_2 is more limiting than light. Still, those isolating plastids from resistant and susceptible strains have noted that there is far less starch in the resistant plastids. Field performance data are available for the triazine resistant rape biotypes that have been backcrossed to the name variety more than 8 times and are thus almost "isogenic" (nuclear). Under comparative hand-weeded conditions the resistant strain yielded less than 85% of the susceptible (I. Morrison & V. Souza-Machado pers. comms. 1983). The weed control problems are often so great in this crop, and the alternative herbicides far less cost-effective than atrazine, that the resistant material is commercially viable. Still this potential yield loss must be considered. As the many triazine resistant biotypes have slightly different properties (e.g. Fig. 4), the effect on photosystem II may be less pronounced in some of them, and they should be used for future breeding work.

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3C—S4

STRATEGIES FOR TESTING AND MANAGEMENT OF FUNGICIDES FOR CONTROL OF MONILINIA IN STONE FRUIT CROPS

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ABSTRACT Brown rot (*Monilinia fructicola* and *M. laxa*) on stone fruits is controlled by fungicide applications during bloom, preharvest, and postharvest. Blossom sprays are timed to coincide with periods when blossom parts are susceptible to infection. Preharvest cover sprays are applied during the last month before harvest. Postharvest fungicide treatments are used for crops held during storage, transit, or ripening. Recently, the number of fungicide applications has increased because of more frequent rains during blossom and harvest. Benzimidazole fungicides, which have been used almost exclusively since 1972, provided excellent disease control until resistant *Monilinia fructicola* was detected in 1977 and *M. laxa* in 1980. To prevent crop losses, orchards with brown rot were monitored yearly starting in 1973 for benomyl-resistant *Monilinia*. In California peach orchard, treatment mixture of benomyl and captan was tested in 1979, but did not delay the increase in established benomyl-resistant populations. Benomyl-resistant isolates competed effectively with benomyl-sensitive isolates in their ability to cause blossom and fruit infection. Benomyl-resistant *M. fructicola* overwintered as mycelium in blossoms and twigs as well as in mummies on the tree. Mummies on the ground produced apothecia with benomyl-resistant ascospores. Alternative fungicides, triforine and iprodione, were introduced in 1981 and 1983, respectively. After two years of exclusive triforine applications in peach orchards, benomyl-resistant populations remained unchanged. With *M. laxa*, the 1980 benomyl-resistant isolates collected from apricot fruits were less fit than sensitive isolates in their ability to cause blossom blight or twig cankers. Although crop losses due to benzimidazole-resistant *M. laxa* have not been reported, in 1983 severe apricot blossom blighting was observed. Isolates from the blighted blossoms revealed benomyl-resistant *M. laxa* and *M. fructicola* in benomyl-sprayed apricot orchards. The management of benomyl-resistant *M. laxa* on apricots could be achieved by use of an eradicant fungicide, such as monocalcium arsenite (no longer registered), applied during the dormant period to eliminate essentially all overwintering inoculum, but the possibility of environmental pollution prevents re-registration of monocalcium arsenite. Programs for brown rot control on stone fruit crops require cooperation and coordination of the pesticide and agricultural industries to promote alternating the use of different fungicides, to require monitoring programs to detect fungicide-resistant *Monilinia* lines, and to minimize application of certain fungicides in order to reduce selection pressures.

INTRODUCTION

Brown rot blossom blight and fruit rot in California are caused by *Monilinia fructicola* and *M. laxa* (Sonoda *et al* 1982). On peaches and nectarines, *M. fructicola* is the primary pathogen whereas on apricot and almond, *M. laxa* is the primary pathogen. Recently, both *Monilinia* species have been found in these crops. Since brown rot-resistant stone fruit cultivars are not available, current disease management strategies focus on cultural practices and fungicide applications. The most important cultural practices are removal of mummified fruit from trees after harvest and the use of low-angle sprinklers to prevent free moisture deposition on blossoms and fruit during the last month before harvest. Fungicides are required to reduce crop losses, but in order to retain their extended useage one must consider that *Monilinia* species have heterocaryotic multinuclear thalli and conidia which make them more prone to selection towards fungicide-resistant lines. The strategies discussed in this paper are: 1) testing procedures of fungicides based on their specific mechanisms of action and the disease cycle of the crop, and 2) management of fungicides to delay the rapid selection of established fungicide-resistant *Monilinia* lines.

The disease control program for brown rot on stone fruits depends on whether the fungicides employed are eradicant, protectant, or suppressant in their activity and on proper timing and application of the chemicals. An eradicant chemical may reduce the primary inoculum or control established infections. However, most available fungicides are protectants, necessitating a strategy that prevents initial infections in order to reduce production of secondary inoculum. For the stone fruit species, phenological stages of blossoming determine the timing of treatments to protect susceptible host parts. For example, on peaches and nectarines, infection occurs almost exclusively through the anthers, whereas all flower parts of prunes and apricots are susceptible to infection. All almond and sweet cherry flower parts except sepals are susceptible to infection (Wilson & Ogawa 1979). Protectant fungicides which prevent initial infections of blossoms may also prevent twig blight. Fungicide applications by ground sprayer are usually highly effective. Aircraft applications are most effective for chemicals with some systemic activity, such as the benzimidazole, benomyl (Yates *et al* 1974). Fungicides do not control preharvest fruit rots resulting from quiescent infections which occurred during the shuck-fall stage of bloom. Maturing fruits become susceptible about a month before harvest. Since at harvest fruits are highly susceptible to infection, mature but not ripe fruits are picked for fresh market. Postharvest treatments control brown rot as well as a complex of other fungal pathogens including *Rhizopus* species, *Botrytis cinerea*, and *Penicillium expansum*. Some chemicals such as DCNA (dicloran) and benomyl can suppress established *Monilinia* and *Rhizopus* infections.

TESTING FUNGICIDES

The fungicides used for brown rot control are classified as eradicants (monocalcium arsenite, sodium pentachlorophenoxide, mercuries, and hypochlorous acid) and protectants such as sulfurs, coppers, dithiocarbamates, captan, dichlone, dicloran, benzimidazoles, sterol inhibitor (e.g., triforine) and dicarboximide (e.g., iprodione). A few of the fungicides mentioned show some degree of systemic activity (benzimidazoles and dicloran) and some have been reported to show suppressant (kickback) action (captan, dichlone, dicloran, and the benzimidazoles). Today the eradicants monocalcium arsenite and sodium pentachlorophenoxide are no longer registered even though they were applied when the host was dormant, resulting in minimal fungicide residues on the fruit. Pathogen resistance to the non-systemic protectant fungicides has not developed but nonsystemics are less effective than the systemic fungicides such as the benzimidazoles. Triforine, a sterol inhibitor, has been proved as an effective alternative to the benzimidazoles but requires more applications. The use of triforine is somewhat limited on crops such as sweet cherries and almonds since it does not control blossom blight caused by Botrytis. Iprodione, a dicarboximide effective against both Monilinia and Botrytis, was used effectively on limited acreage during the 1983 season. Sterol inhibitors which may soon be registered for use on stone fruits include CGA 64251 and prochloraz. DuPont's experimental DPX-H 6573 and the dicarboximide vinclozolin also show potential for control of brown rot.

MANAGEMENT STRATEGY

An understanding of the series of events beginning with the release of benomyl for experimental use in 1967 and leading to widespread M. fructicola and M. laxa resistance to benomyl is necessary to plan strategies to manage new fungicides for control of brown rot. Laboratory tests in 1967 established a baseline sensitivity of 1.0 µg/ml for the two brown rot fungi. In field tests, benomyl controlled blossom blight and fruit rot of stone fruits at concentrations less than 300 µg/ml (Ogawa et al 1968). Resistance to benomyl was first reported for cucumber powdery mildew in the greenhouse in 1969 (Schroeder and Provvidenti 1969) which was a signal that other fungi can also develop resistance to benomyl. Ramsdell & Ogawa (1973) demonstrated systemic activity of benomyl on almond blossoms and the need for a single spray for an effective control. Benomyl was registered for commercial use on stone fruits in 1972. Surveys in California orchards in 1973 and 1974 failed to detect benomyl resistant lines of Monilinia (Tate et al 1974). During this period the label recommendation suggested repeated applications of benomyl on stone fruits. An article written for growers by the manufacturer of benomyl stated that if another fungicide was used at bloom stages, preharvest benomyl sprays should not be used, thus prompting growers to use benomyl exclusively (E.I. du Pont de Nemours and Co., Inc. 1973). After benomyl-resistant M. fructicola was first reported from Australia (Whan 1976) and later from Michigan (Jones & Ehret 1976) and New York (Szkolnik & Gilpatrick 1977), information on procedures for detection of Monilinia resistant to benomyl was made available in the United States (Ogawa et al 1977, Ogawa et al 1978, Ogawa et al 1983a) and internationally (Ogawa et al 1979). In California, information supplied to the state by the manufacturer of benomyl (Delp 1980, E.I. du Pont de Nemours and Co., Inc. 1977) resulted in a mandatory requirement for mixture treatments of benomyl plus a protectant fungicide. In 1977, after five years of almost exclusive commercial use of benomyl, resistant lines of M. fructicola were detected in a peach orchard in California, and by 1982 resistance was widespread. Benomyl sprays became ineffective in orchards which had large populations of 1-4 µg/ml benomyl-resistant M. fructicola, but where the resistant population was small, benomyl continued to control brown rot (Sonoda et al 1983). In a California peach orchard, benomyl-captan mixture treatments did not result in a smaller population of M. fructicola than treatment with benomyl alone (Szkolnik et al 1978); additionally, benomyl-captan sprays were no more effective for brown rot blossom blight control than benomyl alone (Dijkhuizen et al 1983). Laboratory studies on the ability of benomyl-resistant M. fructicola isolates to compete with benomyl-sensitive isolates showed that the sensitive isolates were somewhat more aggressive in mycelial growth (Sonoda & Ogawa 1982). Benomyl-resistant and benomyl-sensitive M. fructicola were isolated from the same blighted blossoms and twigs, mummies on the tree, and apothecia (Shabi & Ogawa 1981). New fungicides such as triforine and iprodione are the only alternatives for control of M. fructicola benomyl-resistant lines, however, resistance to these chemicals has been shown in laboratory tests (Gilpatrick 1981, Katan & Shabi 1981, Sztenjberg & Jones 1978). Isolations from orchards where triforine replaced benomyl showed that changes in populations of benomyl-resistant lines did not occur for a two-year period.

Benomyl-resistant isolates of M. laxa were not found until 1980 (Ogawa et al 1983). The first benomyl-resistant isolates of M. laxa were found less fit in their ability to produce blossom blight and twig cankers on almonds and prunes (Canez & Ogawa 1982). Disease control failure attributed to benomyl-resistant M. laxa was not found until the spring of 1983. The 1983 failures in blossom blight control on apricots and almonds sprayed with benomyl suggest that M. laxa resistant lines compete effectively with sensitive lines and will be a problem unless alternative fungicides are used.

The management strategy for new fungicides is to establish a baseline sensitivity level (minimum inhibitory concentration required for mycelial growth and/or ED_{50} values for mycelial growth) for a large population of Monilinia collected before the fungicide is registered for commercial use. Once the chemical is used commercially, orchards must be monitored for fungicide-resistant lines, especially where disease control has failed (Ogawa et al 1981). The number of fungicide applications should be minimized because repeated applications provide pressure for selection of resistant Monilinia. In order to keep the inoculum level low, the most effective systemic fungicide available should be used for the first spray of the season. The second application could be a fungicide with suppressive action; additional cover sprays could be fungicides not known to select for fungicide-resistant lines. Mixtures of chemicals combining a highly effective systemic fungicide such as benomyl with currently available moderately effective fungicide, such as captan, ziram, or maneb, are not recommended to prevent or delay the development of resistant Monilinia. Unless chemical mixtures are used to control more than one pathogen or disease, they are expensive when used at full dosage and may promote excessive use of pesticides.

A model fungicide management schedule to control brown rot of stone fruits in California requires the application of at least two unrelated protective fungicides as well as the use of an eradicant dormant fungicide (Table 1). The timing of sprays using these fungicides is based on specific inherent qualities such as their systemic activity, suppressive activity (kickback action), and plant growth stage during which the chemical protects against infection. Since new fungicides could select for resistant lines of Monilinia, the strategy to prevent or delay the development of resistance involves alternating sprays of unrelated fungicides. We have assumed in this fungicide management program that dormant eradicant fungicides are available, resistance problems are not expected for the older nonsystemic fungicides, and that a systemic fungicide, similar to the benzimidazole fungicide in effectiveness, could select for resistant Monilinia.

For crops such as almond, apricot, and prune an eradicant fungicide applied during the dormant stage would reduce or almost eliminate the inoculum source of M. laxa. Thus the number of blossom sprays required would be one, or a maximum of two, and preharvest sprays would not normally be required. If fungicide resistant lines did appear, the dormant eradicant fungicide would reduce the primary inoculum to a sufficiently low level that a single highly effective systemic fungicide application would effectively control the blossom blight. If another spray were required, an unrelated less effective protective fungicide would provide control.

On other stone fruit crops, where an eradicant fungicide has never been available and cover sprays are required during bloom and preharvest, the first protective spray is applied when the first susceptible blossom parts emerge (petals). The fungicide used for this first spray should be systemic in order to obtain protective action on other floral parts as blossoms open. The second spray should be the new alternative fungicide, which may or may not select for resistance, older fungicides which are not known to select for resistant lines of Monilinia are used for all subsequent cover sprays during bloom. The systemic fungicide likely to select for resistant lines could be used again for the first preharvest cover spray, since it should be applied when the inoculum level is at its minimum and therefore the chances of selection for fungicide- resistant lines would be the least. The second preharvest spray could utilize the alternative fungicide with an unknown record for selecting fungicide-resistant lines, and again an older fungicide would be used for subsequent cover sprays. Spray mixtures are considered for multiple field sprays when other diseases (Table 1) require control. Mixtures of two unrelated spray chemicals can reduce the chances of a disease epidemic resulting from resistant lines developing for one or the other chemical if both selected fungicides can effectively control the brown rot disease. Multiple site mode of action fungicides would appear to best prevent or delay the development of resistant lines of Monilinia.

Postharvest treatments are required for fresh market fruit and must utilize the most effective brown rot control fungicide available. If this chemical has systemic activity and could select for resistance, it should be reserved, if at all possible, for postharvest use. Stone fruits treated with fungicides are not likely to select for resistant lines since they are commonly shipped and consumed without extended storage periods. However, treated fruits which are rejected should be destroyed to prevent possible resistant pathogens from contaminating marketable fruits during packing and storage. In order to prevent postharvest losses, two or more fungicide mixture treatments are suggested to control brown rot as well as a complex of decay pathogens. The use of an eradicant fungicide such as hypochlorous acid in preliminary wash water to remove surface contaminants from the fruit, is highly recommended to provide more efficacious decay control with protective fungicides.

Some of the difficulties in developing a sound disease management program include the decrease in numbers of available fungicides and lack of information on the optimal way to use individual fungicides. The conditions that promote selection of Monilinia lines which are resistant to fungicides and which compete effectively with the sensitive lines are not understood, although there is evidence that the first detected resistant isolates were not as fit for survival as subsequent resistant isolates. Preharvest disease managment programs are weakened by the difficulty of obtaining a regulation prohibiting the exclusive use of certain fungicides. Reports of laboratory-induced resistance should prompt adoption of new strategies for fungicide usage in the field, especially to insure maximum efficacy and life expectancy for the newly introduced sterol inhibitors and dicarboximides, since they control brown rot more effectively than older fungicides.

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TABLE 1

Model fungicide control strategy for control of brown rot on stone fruits and nuts in California

		Fungicide treatments						
		Pre-	5%	Full	Preharvest (da)			
Crop	Dormant	bloom	bloom	bloom	21	14	7	Postharvest
Almond	E[a]	--	SP_1[b]	$NP_{1,2}$	--[c]	--	--	--
Apricot	E	SP	NP_1	$NP_{1,2}$	?[d]	?	?	$SP+NP_{3,4,5}$
Prune	E	SP	NP_2	$NP_{2,5}$	?	?	?	--
Cherry	--	--	SP_2	NP_2	?	?	?	$E+SP+NP_{3,4,5}$
Nectarine	--	--	SP	NP_1	SP	NP	NP	$E+SP+NP_{3,4,5}$
Peach	--	--	SP	NP_1	SP	NP	NP	$E+SP+NP_{3,4,5}$
Plum	--	SP	NP_2	NP_2	?	?	?	E+SP+NPe,r,t

[a]Fungicide designations: E = eradicant; SP = systemic protectant; and NP = nonsystemic or new protectant.

[b]Other pathogens or disease: 1 = shot hole; 2 = gray mold; 3 = bread mold; 4 = blue-green mold; and 5 = russet scab disease.

[c]Treatment not used.

[d]Effectiveness of treatment not verified.

THE DYNAMICS OF TRIAZINE-RESISTANT WEED POPULATIONS

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Background and objectives

Evolution of resistance to triazine herbicides in populations of weed species was first recorded during the late 1960's in the United States of America. Resistant populations have become widespread in maize crops in North America and continental Europe and also occur in orchards, vineyards and tree nurseries. Although triazine herbicides have been used in the United Kingdom for 20 years, triazine-resistant populations of weeds only started to appear in the 1980's. The majority of resistant populations occur in orchards, ornamental plant nurseries and soft fruit crops where simazine has been used extensively and repeatedly. The global common factor which determines the evolution of resistance has been the recurrent application of relatively persistent chemicals (e.g. atrazine or simazine) on the same fields for at least five years and usually longer (8-12 years).

Our field experiments investigated the nature of the selective forces leading to evolution of resistance in *Senecio vulgaris*. The investigation had two major concerns. These were first, a study of intensity and duration of selection and genetic variability in target populations of weed species and second, a study of the dynamics of resistance frequency in relation to selection pressure and the relative fitness of resistant and susceptible phenotypes in herbicide treated and untreated locations.

Materials and methods

The dynamics of mixed populations of simazine-resistant and susceptible phenotypes of *S. vulgaris* were studied for a period of two years in experimental blackcurrant plantations. Seed mixtures which consisted of 2% resistant phenotypes were sown at an initial density of approximately 100 seeds m^{-2}. The fates of emerged seedlings were followed at two week intervals throughout each year, in plots where there were four kinds of weed control. The treatments were (a) early spring simazine application; (b) spring application of simazine followed by paraquat spot treatment in July; (c) spring application of simazine followed by an autumn application; and (d) no simazine applied; late winter paraquat followed by rotovation in early spring.

Relative fitness of resistant and susceptible phenotypes was assessed in replacement series competition experiments in the glasshouse and in a field experiment where the fates of known resistant and susceptible individuals were followed in the presence and absence of interspecific competition.

Results and conclusions

Life tables were made for cohorts of plants under the four management regimes. In unsprayed plots the seedlings of only one cohort per year (which arose in April/May) survived to disperse fruits. In the second season the population consisted only of susceptible phenotypes. Cohorts of seedlings which emerged from June onwards failed to survive to maturity due to interspecific competition from the weed flora together with infection by *Puccinia eagenophorae*. In the simazine treatments (spring only or spring and autumn), seedlings emerging in spring (April/May) survived to disperse fruits in summer but some autumn recruits (September/October) grew into adults that overwintered and dispersed fruits in the following late spring.

The herbicide exerted intense selection since in the first season the initial seedling cohort comprised 89% resistant phenotypes in contrast to 0.5% resistance in the control (rotovated) treatment. However there were apparently a few susceptible escapees since there were 7% susceptible progeny in the 1979 dispersed seed. In the second season the April seedling population consisted of 96% resistant phenotypes. Spot treatment of paraquat in July did not prevent an increase in the percentage of resistant phenotypes but there was a much greater proportion of susceptible phenotypes (50%) in progeny from the summer seedling cohort. The herbicide programme was clearly important in determining the genetical structure of *S. vulgaris* populations.

In competition, the relative fitness of resistant phenotypes was 0.85 in relation to susceptible phenotypes. In a non-herbicide environment the proportion of resistant phenotypes in the population would gradually decline.

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3C—R2

COMPARATIVE FITNESS OF NATURALLY OCCURRING DICARBOXIMIDE-RESISTANT STRAINS OF *BOTRYTIS CINEREA* PERS.

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Background and objectives

Strains of *Botrytis cinerea* Pers. resistant to dicarboximides are present in Italy in some protected crops (i.e. tomato, lettuce) and, at a lower frequency, on grape. Contrary to what happened in other mediterranean countries (Katan, 1982; Leroux, 1983), dicarboximides are still effective in controlling gray mould in Italy. Probably this is due to the fact that naturally occurring dicarboximide-resistant strains of *B.cinerea* show a relatively low level of resistance if compared to resistant strains selected *in vitro*.

We sought to evaluate the comparative fitness of these resistant strains and their behaviour, under greenhouse conditions, in mixed populations with sensitive strains with and without selection by dicarboximide-fungicides.

Materials and Methods

The comparative fitness of several naturally occurring dicarboximide-resistant strains of *B.cinerea* isolated from grapes and tomatoes was evaluated by means of successive transfers of mixed populations of sensitive and resistant conidia (50:50 initial ratio) of the different strains on fungicide-free medium and on untreated grape berries. The behaviour of mixed populations under greenhouse conditions was studied on tomato plants artificially inoculated with mixtures of sensitive and resistant strains and sprayed with vinclozolin weekly, every three weeks, or weekly with dichlofluanid.

Results and conclusions

In Petri plates resistant strains of *B. cinerea* seemed very fit and able to survive. But in grape berries, even after 3 transfers, the percentage of resistant conidia decreased sharply, showing a reduced fitness of resistant strains in conditions resembling those occurring in the field (Romano *et al.*, 1983). This can probably be explained by the slightly reduced virulence of the majority of the resistant strains compared to the sensitive ones, which delays infection caused by dicarboximide-resistant strains.

In greenhouse experiments, weekly sprays with vinclozolin could control gray mould caused by the mixed population of sensitive and resistant strains, while sprays carried out weekly with dichlofluanid or every three weeks with vinclozolin were less effective in controlling the pathogen. Only dicarboximide-resistant strains could be isolated from infected tomato plants treated with vinclozolin. However few resistant strains were reisolated from rotted fruits collected from untreated plots 2 months after inoculation. This suggests that resistant strains declined in mixed populations in the absence of the dicarboximides. Those able to infect tomato fruits and to survive under natural conditions were generally at least partially controlled by dicarboximides under our experimental conditions (Romano *et al.*, 1983).

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INFLUENCE OF VINCLOZOLIN MIXTURES ON THE DEVELOPMENT OF RESISTANCE AND ON DISEASE CONTROL IN BOTRYTIS CINEREA PERS. OF GRAPES

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Background and objectives

Since 1979 resistance of Botrytis cinerea on grapes to dicarboximides has spread widely over all the vine-growing areas in Western Germany which in previous years had been treated with these fungicides (Lorenz et al 1981). The proportion of resistant Botrytis strains in the total Botrytis population normally varies between 50% and 80%, but decreases during the time when treatments are not applied - after harvest until the beginning of the next growing period - only to rise again when dicarboximide spraying begins.

In 1979, soon after the first dicarboximide resistant Botrytis strains were discovered in the field (Holz, 1979), trials were initiated with the aim of trying to prevent the increase and spread by using particular spraying programmes. The results of these trials are reported here.

Materials and Methods

Trials were situated in the Palatinate and Mosel wine-growing areas. In 1979 one trial was assessed, in 1980 two, in 1981 five, and in 1982 three. Plot size was 20-30 vines, with 2-4 replications. Treatments were carried out using knapsack sprayers with a spray volume of 2,000 l/ha. Botrytis was assessed on 6 x 100 grapes, which were divided into categories 1-6 (1 = no disease to 6 = > 50%), and the disease intensity calculated. To determine the sensitivity of B. cinerea strains to dicarboximides samples were taken from 5-8 positions in the plots, as follows: in February wood, in June inflorescences and leaves, and in October, shortly before harvest, grapes and leaves. These were investigated in the laboratory. 10-12 Botrytis isolates from each plot and from each sampling position were tested in agar diffusion tests using filter paper discs to determine their reactions to vinclozolin.

Results and conclusions

In 1979 Botrytis was not very severe in control plots - (15%, AB, Duncan Test *). After sprays at growth stages 17, 25, 32, 34 and 35 (according to Eichhorn & Lorenz 1977), disease infection was reduced to 8% (A) with vinclozolin (1 kg a.i./ha), to 4% (A) with dichlofluanid (2 kg a.i./ha) and to 5% (A) with thiram (3.2 kg a.i./ha). Mixtures of vinclozolin (1 kg a.i./ha) with either dichlofluanid (2 kg a.i./ha) or thiram (3.2 kg a.i./ha and 1.6 kg a.i./ha) brought no further improvement (3-7%, A).

Botrytis in 1980 was more severe than in 1979 with 30% (C) infection in control plots. Five treatments with vinclozolin (1 kg a.i./ha), at the same growth stages as in 1979, reduced the disease level to 16% (AB). The mixtures vinclozolin+dichlofluanid and + thiram (lower rates than in 1979) were less effective than vinclozolin alone (21%, ABC and 25%, BC respectively). Mixtures of vinclozolin (1 kg a.i./ha) with either captan (1 kg a.i./ha) or chlorothalonil (1.5 kg a.i./ha) improved the effectiveness and infection levels were 13% (A) and 11% (A), respectively. Similar results were obtained in the second trial.

At the end of September 1981 9% (D) Botrytis was found in control plots. Four treatments with vinclozolin (1 kg a.i./ha), at growth stages 25, 32, 34 and 35 were without-effect (8%, D). With chlorothalonil (3 kg a.i./ha), dichlofluanid (2 kg a.i./ha) and captan (2 kg a.i./ha) infection levels were between 3 and 4%. Of the mixtures of vinclozolin (1 kg a.i./ha) with chlorothalonil (1.5 kg a.i./ha and 3 kg a.i./ha), with dichlofluanid (1 kg a.i./ha and 2 kg a.i./ha) and with captan (1 kg a.i./ha and 2 kg a.i./ha), only the tank mix with chlorothalonil produced a significant improvement in control (2%, A), at both the high and low rates. These results were confirmed in 3 further trials but in another trial with a higher disease level (30%) there was no significant difference between the treatments.

In 1982, when infection in control plots was 43% (E) results were similar to those obtained in 1981. After four sprays with vinclozolin (0.75 kg a.i./ha) or myclozolin (0.5 kg a.i./ha) disease levels were 35% (CDE) and 38% (DE) respectively. Mixtures of myclozolin (0.5 kg a.i.ha) with dichlofluanid (0.5, 0.75 and 1 kg a.i.ha) were scarcely better than myclozolin alone. Tank mixes of myclozolin (0.5 kg a.i./ha) with chlorothalonil (0.5, 0.75 and 1 kg a.i./ha) reduced infection levels to 24% (AB) and 19% (A) respectively. Reducing the rate of myclozolin to 0.33 kg a.i./ha in mixtures with the three chlorothalonil rates produced similar disease levels. Results of two further trials confirmed these findings.

Laboratory investigations accompanying these trials showed repeatedly that none of the mixtures prevented the increase in resistant strains which occurred during the spraying period. Results from glasshouse-experiments using B. cinerea on Pelargoniums confirmed that a mixture of dicarboximide with chlorothalonil proved better when resistance was already present than dicarboximide alone, or any of the other mixtures tested.

The biological effect against dicarboximide resistant Botrytis strains was, in general, not better with mixtures of vinclozolin with either thiram, dichlofluanid or captan than it was with vinclozolin alone. The tank mix of vinclozolin with chlorothalonil gave better disease control than both the above mixtures and vinclozolin alone. No effect of mixtures on the proportions of sensitive and resistant isolates could be detected.

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* Data with the same letter(s) in common are not significantly different.

THE EFFECT OF DICARBOXIMIDE FUNGICIDES ON SCLEROTIUM CEPIVORUM

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Background and objectives

Combined seed and stem base treatment with iprodione has given effective control of Allium white rot for several years (Entwistle & Munasinghe, 1982). In 1981/1982, however, iprodione failed to control white rot in experiments with overwintered onions at the NVRS. S. cepivorum isolates from infected onions, and from stock cultures, were tested for their in-vitro response to iprodione and to the chemically related compounds vinclozolin and meclozolin (myclozolin).

Materials and Methods.

Field plots were infested with sclerotia of iprodione-sensitive isolates of S. cepivorum (Entwistle & Munasinghe, 1982). Salad onions were sown in spring 1982 and assessed for white rot symptoms at intervals until autumn and the effect of iprodione compared with vinclozolin and meclozolin. Salad onions were also sown in autumn 1982, treated with iprodione, and white rot isolates tested on malt agar containing 5 ppm iprodione. These and stock isolates were tested on malt agar containing either 5 ppm vinclozolin or meclozolin.

Results and conclusions

Vinclozolin and meclozolin seed (50g a.i./kg seed) and stem base (0.05g a.i./m row) treatment effectively controlled the disease whereas iprodione was ineffective (90% white rot). Mycelium from iprodione-treated onions grew normally on malt agar but failed to grow normally on iprodione agar. Of 173 mycelial and sclerotial isolates tested from infected iprodione-treated onions, four formed tufts of aerial hyphae growing away from the agar; one grew normally (25 mm/d); and the remainder failed to grow on iprodione agar. These tufted isolates grew normally on fungicide-free agar, but when returned to iprodione agar, sometimes failed to grow. The one isolate that grew normally on iprodione agar occasionally formed dark, slow growing (1 mm/d) sectors. Thus, S. cepivorum is either iprodione sensitive (no growth) or resistant (dark slow-growing or normal colonies), although this resistance is not always stable.

TABLE 1

Response of S. cepivorum to three dicarboximide fungicides

Isolate	Iprodione			Vinclozolin			Meclozolin		
	No growth	Dark slow growing	Normal	No growth	Dark slow growing	Normal	No growth	Dark slow growing	Normal
1	+	-	-	+	-	-	+	-	-
2	-	+	-	-	+	-	-	+	-
3	-	-	+	-	-	+	-	-	+
4	+	-	-	-	+	-	+	-	-

Iprodione resistant isolates showed a similar response to vinclozolin and meclozolin (5 ppm Table 1). However, isolates forming dark slow-growing colonies on vinclozolin agar did not always show the same response to other dicarboximides. Like iprodione, vinclozolin resistance was also unstable. One colony that grew normally on vinclozolin agar, produced dark slow-growing colonies when subcultured onto fresh vinclozolin agar, and very little growth on iprodione or meclozolin. Growth ceased altogether when this isolate was subcultured a second time onto vinclozolin agar. One isolate, however, continued to grow normally in the presence of each dicarboximide after two subcultures.

S. cepivorum, whilst generally sensitive to the in-vitro presence of dicarboximide, can become resistant. This capacity is unstable and does not appear to account for the failure of control in field conditions.

References

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3C—R5

RESISTANCE IN BOTRYTIS SQUAMOSA TO IPRODIONE

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Background and objectives

The development of benomyl resistance in B. squamosa (onion leaf rot) led to a loss of disease control in onion crops sprayed with that fungicide (Presly & Maude 1980). The present study examines the occurrence in vitro of resistance in B. squamosa to iprodione and its possible implications for disease control in onion crops.

Materials and Methods

A total of 47 B. squamosa isolates were collected from three areas in Britain. Resistant isolates were produced in vitro by selection on Coons agar containing 4-2,500 µg/ml of iprodione. The location of iprodione resistant mycelium within otherwise sensitive fully grown cultures from either single or mass spore isolates was established as follows. Ninety-three discs, (6 mm diam.), were removed from each plate. Each disc was identified positionally and grown on agar containing 4 µg/ml iprodione.

Pathogenicity tests were conducted by spraying spores (5 ml of 0.1 x 10^6 spores/ml/treatment) onto onion seedlings, half of which were sprayed with iprodione (0.05% a.i., 'Rovral' w.p.). Lesion counts were made after 3 days incubation at 15°C and 100% RH. Leaves were surfaced-sterilised in 10% chloros (1.1% free chlorine) for 30 s, washed in sterile distilled water and incubated for 48 h to encourage mycelial growth and sporulation.

Results and conclusions

Iprodione resistant isolates were produced by 10 of the 47 isolates at all concentrations of iprodione. The numbers of resistant isolates produced varied and depended upon the genetic variability of the parental culture. Few discs from cultures of single spore origin produced resistant colonies (0.28%) whereas discs from cultures of multispore origin gave 17-20% resistant colonies on agar containing 4 µg/ml iprodione.

Resistant mycelium occurred at random within otherwise morphologically indistinguishable sensitive cultures. Such mycelia exhibited widely different growth rates and some occurring near the centre of plates were occluded by faster growing sensitive mycelium.

Iprodione resistant isolates were stable but not as fit in pathogenicity tests as were sensitive wild types. Spores produced by some were virtually non-pathogenic causing few lesions while others were of intermediate pathogenicity. Some, especially on iprodione treated leaves, produced almost as many lesions as sensitive isolates on untreated leaves. Pathogenicity was not determined by the concentration of iprodione on which resistant isolates arose.

Surface sterilisation, eradicated the fungus from many lesions caused by resistant isolates; those produced by sensitive isolates were less affected. This indicated a lower rate of infection from resistant spores. Sporulation by resistant isolates took longer, spores appearing 24-48 h later than on leaves sprayed with sensitive isolates. It appears, therefore, that although iprodione resistant isolates are produced readily in vitro many are non pathogenic. Others may infect and sporulate on onion leaves but take longer than sensitive isolates.

Unlike benomyl resistance in B. squamosa, which has been found in the laboratory and the field, iprodione resistance has only been found in the laboratory. This may be related to the reduced pathogenicity of iprodione resistant isolates and indicates that the fungicide may continue to give effective control in field crops. A similar situation has been reported in field isolates in respect of the occurence of iprodione resistance in B. cinerea (Pommer & Lorenz 1982). This type of resistance, however, was identified in vitro in 1961 where B. allii produced a similar response to dichloronitroaniline fungicides which are related to the dicarboximides (Priest & Wood 1961).

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EFFECTS OF DIFFERENT SPRAY REGIMES ON DICARBOXIMIDE RESISTANCE IN BOTRYTIS CINEREA ON STRAWBERRIES

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Background and objectives

The increase in fungicide-resistant forms of pathogenic fungi in crops is generally considered to depend greatly on the nature of the fungicide treatments applied. For example, repeated sprays of the same fungicide are thought to encourage resistance, and application of mixtures or alternating treatments of two with different modes of action to delay it. Opinions differ on whether increasing the dose enhances or hinders resistance. However, there is little experimental evidence comparing the effects of different spray regimes.

Forms of Botrytis cinerea resistant to the dicarboximide fungicides (e.g. iprodione, procymidone, vinclozolin) have been found in strawberries and other crops, and have affected disease control in some situations where many sprays of these fungicides have been applied (see Beever and Byrde, 1982). The response of B. cinerea populations to fungicides is a convenient system for studying the resistance phenomenon, and we have investigated effects of different treatments of procymidone and the unrelated fungicide dichlofluanid on the incidence of dicarboximide-resistant variants of B. cinerea on tunnel-grown strawberry plants cv. Cambridge Vigour.

Materials and Methods

A half-strength spray of procymidone (50% w.p.) was applied to all plants in early March, with the aim of increasing the initial frequency of resistant forms in leaf debris. After one month, this was examined by transferring single conidiophores onto agar containing iprodione at 2 or 100 µg/ml and measuring growth after 4 d at 25°C. Three sprays were applied during flowering (late April - May) at 10-day intervals, to triplicate 9-plant plots. Programmes were: procymidone only, at double, normal (500 mg a.i./l) and half strength; dichlofluanid (50% w.p.) at normal strength (1000 mg a.i./l); tank mixes of the two (at normal and half strength) and an alternation of procymidone-dichlofluanid-procymidone at normal rates. All infected fruits were collected in late May and early June and the fungus isolated and tested as above.

Results and conclusions

In early April, 23% of isolates ('resistant' isolates) grew well with 2 µg/ml (but not on 100 µg/ml) iprodione; the remainder ('sensitive' isolates) did not grow with 2 µg/ml iprodione. All treatments gave good disease control (71 - 90%). Whenever procymidone was applied (alone, in mixture or alternated) isolates were mostly (> 85%) resistant. The proportion of resistant forms changed little with dichlofluanid alone (25%), but declined to 5.7% in totally untreated plots. Debris sampling in July showed large proportions of resistant forms in many plots, again greatest wherever procymidone had been used. However, by January 1983 no isolate tested from any plot was resistant.

The resistant forms appear to require sustained selection pressure if they are to become and remain dominant. Use of a mixed treatment or alternating programme did not prevent the increase in resistance, even though each component alone gave very good control of the total population and absolute amounts of sensitive plus resistant forms decreased. In this experiment the initial proportion of resistant forms was substantial (23%). The experiment is being repeated in 1983, but from a starting population in which resistant forms are scarcer, and it is hoped to present results at the Congress.

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3C—R7

METALAXYL RESISTANCE AND CONTROL OF CUCUMBER DOWNY MILDEW (*Pseudoperonospora cubensis*)

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Background and objectives

Following severe outbreak of downy mildew infections probably resulting from the development of resistance to metalaxyl in *P. cubensis* (Pappas, 1980), surveys were undertaken in protected cucumber crops to ascertain infection by resistant strains. In this respect, a number of new fungicides were tested against resistant and sensitive isolates of the pathogen.

Materials and Methods

P. cubensis isolates were established onto potted cucumber plants (hybrid Dyanna), maintained in a growth chamber (20^{0}C, 60-70% r.h., 12h lighting) under separate polyethylene bags. Sensitivity to metalaxyl was determined as follows: Excised cucumber leaves were floated with the lower surface in contact with four different aqueous fungicide solutions in Petri-dishes (10ml). Distilled water was used as control. After 24h leaves were inverted and when dry inoculated with 10 droplets of sporangial suspension (50,000/ml). Infection was assessed 5 days after inoculation by counting the number of lesions per leaf (0-10).

Protective and curative properties of fungicides were tested following application to foliage of potted plants using a spray gun. Disease control trials were carried out in a polyethylene tunnel (15x5x2.3 m). Fungicide sprays began at the 3 leaf stage (48h before the inoculation of the first leaf) and were repeated weekly until the 25 leaf stage (5-6 sprays). A knapsack sprayer was used for these applications. Disease was assessed after the last spray.

Results and conclusions

Widespread distribution of metalaxyl-resistant strains of *P. cubensis* occurred in cucumber greenhouses regardless of the fungicides used. Resistant isolates infected cucumber leaves floated on solutions of 250 µg metalaxyl/ml, whereas sensitive isolates were inhibited at concentrations of 10 µg/ml. Metalaxyl gave no protection against resistant strains on potted plants, and against sensitive strains its protective effects did not exceed three days.

TABLE 1
Control of downy mildew on cucumber plants grown in a polyethylene tunnel

Treatment	Concn (a.i.%)	Inoculum: Me-sensitive (1981) Infection[1] (%)	Me-sensitive (1981) Sensitivity[2]	Inoculum: Me-resistant (1982) Infection[1] (%)	Me-resistant (1982) Sensitivity[2]
Cymoxanil (C)	0.05	49.7 f	1.13	19.1 c	6.25
Fosetyl-Al (F)	0.20	14.9 cd	0.38	7.1 b	6.50
Metalaxyl (Me)	0.025	17.4 d	0.38	55.0 e	7.00
Mancozeb (Ma)	0.20	10.5 bcd	0.00	0.1 a	6.75
(C + Ma)	0.01+0.16	5.5 ab	-	0.1 a	-
(F + Ma)	0.15+0.09	1.8 a	-	0.1 a	-
(Me + Ma)	0.015+0.11	2.0 a	-	0.8 a	-
Me altern. with Ma		28.7 e	-	7.8 b	-
(Me + Ma) altern. with Ma		3.8 a	-	0.8 a	-
Untreated	-	67.2 g	0.25	39.2 d	9.25
Mother isolate			0.13		7.50

Data followed by the same letters do not differ significantly at $\underline{p}=0.05$.
[1]Infection assessed using the scale: 15=1-30; 40=30-50; 60=50-70; 85=70-100% leaf area infected. Each figure is the mean infection of 9 plants.
[2]Mean infection of 4 leaves floated on different metalaxyl concentrations (10, 50, 100, 250 µg/ml). Each figure is the mean of 3 isolates sampled after the last spray.

In polyethylene tunnel trials mixtures of each of the three specific fungicides with mancozeb gave better control than the fungicides alone. Even metalaxyl-resistant strains were controlled with a metalaxyl + mancozeb mixture (see Table 1).

However, none of the specific fungicides were particularly effective against *P. cubensis*, and control of the disease should be based on prophylactic spray programmes which include conventional fungicides. These schedules may also minimize the risk of control failure through development of resistance.

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BENZIMIDAZOLE ('MBC') RESISTANCE IN PSEUDOCERCOSPORELLA HERPOTRICHOIDES

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Background and objectives

Carbendazim resistant conidia of the eyespot fungus (Pseudocercosporella herpotrichoides) were detected in Germany but, until 1983, their frequency was low and considered to be of no practical significance. The objectives of this study were to investigate reported failures of 'MBC' fungicides to control eyespot in two winter wheat crops in England in 1981, to examine certain characteristics of 'MBC' resistant strains and to check for their presence in fields elsewhere in England.

Materials and Methods

In 1982, unreplicated fungicide trials were undertaken in two winter wheat crops. At both sites, 'MBC' fungicides had given poor control of eyespot in 1981 when 'MBC' resistant strains were isolated. Mycelial isolates of P. herpotrichoides were obtained from plots at both sites and from 59 randomly selected fields of winter wheat and winter barley. Previous use of 'MBC' fungicides during the period 1975-81 was recorded for 57 sites.

Eyespot fungus was isolated onto a medium lacking fungicide, subcultured on potato dextrose agar (PDA) and incubated in the dark at 20°C. Resistance tests were made by transferring mycelial plugs to PDA plates containing 1 $\mu g\ ml^{-1}$ carbendazim or 2 $\mu g\ ml^{-1}$ benomyl; 'MBC' resistant strains grew normally and 'MBC' sensitive strains were completely inhibited.

Dosage response curves were determined with a range of carbendazim concentrations from 0.0-0.5 $\mu g\ ml^{-1}$ for 'MBC' sensitive isolates and from 0-1000 $\mu g\ ml^{-1}$ for resistant isolates. Cross resistance tests examined mycelial growth on PDA plates amended with equimolar concentrations (0-100 μM) of benomyl, carbendazim, thiabendazole, thiophanate-methyl or prochloraz. A few isolates were induced to sporulate, and the resistance of their conidia was checked. PDA plates amended with carbendazim (0-100 $\mu g\ ml^{-1}$) were seeded with spore suspensions (2-5 x 10^2 conidia ml^{-1}) and the number of colonies counted after 7 d incubation at 20°C. Pathogenicity of some 'MBC' sensitive and resistant mycelial isolates was determined on wheat cv. Armada and on rye cv. Dominant.

Results and conclusions

'MBC' fungicides gave no control in the two trials whereas prochloraz reduced eyespot. Most isolates (89-100%) from plots sprayed with an 'MBC' fungicide at GS 30-31 were 'MBC' resistant; only 38-50% were resistant from unsprayed plots. It was concluded that poor performance of 'MBC' fungicides at these sites in 1981 and their failure to control eyespot in 1982, were due to fungicide resistance. In random surveys, 'MBC' resistant mycelial isolates of P. herpotrichoides were found in 40% of 15 winter wheat fields and in 39% of 44 winter barley fields. There was a significant ($P = < 0.01$) positive correlation between the probability of detecting 'MBC' resistance and the number of previous consecutive years in which 'MBC' fungicides were applied to cereals.

Mycelial growth of sensitive isolates on amended PDA was completely inhibited at 0.1-0.5 $\mu g\ ml^{-1}$ carbendazim whereas some resistant isolates still grew at 1000 $\mu g\ ml^{-1}$ carbendazim. Carbendazim resistant isolates were cross resistant to benomyl, thiophanate-methyl and thiabendazole, but not to prochloraz. The sensitivity of conidia to carbendazim was the same as the sensitivity of parental mycelial isolates. 'MBC' resistant isolates were just as pathogenic to wheat as sensitive isolates.

Isolates could be separated into two distinct types based on growth rate and colony morphology. The faster growing darker colonies with regular margins conformed with the W-type (pathogenic to wheat and barley) and the slower growing pale colonies with feathery margins conformed to the R-type (pathogenic to wheat, barley and rye, Scott et al 1975). 'MBC' resistant strains of both types were found. The majority of 'MBC' sensitive isolates were W-type whereas the majority of 'MBC' resistant isolates were R-type. Tests with 'MBC' resistant R-types confirmed that they were pathogenic to rye.

'MBC' resistant strains appear well fitted and have a high resistance factor (> 100). Prochloraz, which has a different mode of action, is an alternative for eyespot control where 'MBC' resistant strains occur.

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3C—R9

EYESPOT CONTROL IN CEREALS WITH PROCHLORAZ AND PROCHLORAZ PLUS CARBENDAZIM

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Background and objectives

Eyespot (Pseudocercosporella herpotrichoides) is a major stem base disease of winter cereals in the U.K. Although husbandry factors and especially choice of cultivar, have a part to play, farmers increasingly depend on fungicides to control eyespot. For over a decade mbc-generating fungicides have been used. However, in 1982, ADAS confirmed the presence of mbc-resistant strains of eyespot, widely distributed throughout the U.K. This has necessitated a re-evaluation of the traditional treatments for control of this disease. Prochloraz 'SPORTAK' introduced in the U.K. in 1980 offers an effective alternative originating from a different chemical group and having a different mode of action. Our trials carried out in 1982 throughout the U.K. examined control of eyespot with prochloraz alone and in mixture with carbendazim 'SPORTAK ALPHA'. This work was extended in 1983 on sites with mbc-resistant and mbc-sensitive strains of eyespot.

Materials and Methods

In 1982 the overall reliability of treatments was assessed in replicated and farmer applied trials. In 1983 the mbc-resistance status of sites was determined in the laboratory before treatments were applied using the mass mycelial isolate technique. From 100 tillers per site, isolations were made on agar (PDA) plates from all tillers showing a convincing eyespot lesion together with a proportion from each site showing some form of lesions or browning. Clean isolates were sub-cultured prior to testing on agar amended with 2 ppm carbendazim or prochloraz. Growth on 2 ppm carbendazim was taken as demonstrating resistance at a level at which eyespot would not be controlled by field applications of mbc. (No criteria exist for establishing the level of prochloraz). Field trials were then carried out on a range of mbc-sensitive and resistant sites widely spread geographically. In both seasons treatments were applied at G.S. 30-31 and replicated six times. Eyespot severity was assessed according to the ADAS scale of disease index (0-3).

Results and conclusions

In 1982 prochloraz + carbendazim (400 + 150 g ai/ha) gave reliable eyespot control with marked improvement over other broad-spectrum products compared in a similar schedule. Prochloraz alone (400 g ai/ha) also gave good control in general but was not quite as reliable as the mixture with carbendazim. Results from the 1983 field programme are not available at the time of writing.

Results from laboratory tests in 1983 involving samples taken from 70 fields before 16th April are shown in table 1.

TABLE 1

In-vitro tests of eyespot sensitivity to carbendazim or prochloraz

No. fields with eyespot	No. isolates tested	Number of isolates resistant to carbendazim (2 ppm)	prochloraz (2 ppm)
31	21	9	0

Trials by Griffin & Yarham in 1982 indicated that prochloraz was effective on mbc-sensitive and mbc-resistant strains of eyespot, in both the laboratory and field. Our own work has confirmed these results. Thus where mbc-resistance has been identified prochloraz offers effective control. Where the resistance status has not been defined prochloraz + carbendazim will ensure a high level of control of eyespot and prevent or slow down the build-up of resistance to straight mbc products. In addition, prochloraz alone or in mixture with carbendazim gives control of a wide range of other pathogens attacking cereals at this time.

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MBC RESISTANCE IN FIELD POPULATIONS OF PSEUDOCERCOSPORELLA HERPOTRICHOIDES

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Background and objectives

MBC-type fungicides have been used in W.Germany for 10 years to control eyespot disease of cereals. The frequency of MBC-resistant strains in natural populations was about 1 in 10^8 (Horsten & Fehrmann 1980), but control problems were not expected as the pathogen spreads slowly, and fungicides were applied only once or twice a year. Nevertheless, a long-term monitoring experiment was started in 1976 to study the development of MBC resistance.

Materials and methods

Wheat was grown continuously at the same site, and eyespot controlled with one carbendazim spray, at usual dosage, applied at GS 31/32. In addition ears were sprayed with MBC or thiabendazole, and, in 1979 and 1980, seed was also treated with carbendazim. Half the 1 ha field remained untreated throughout. Resistance was evaluated by drawing straws at GS 75. After isolating the fungus the frequency of MBC-resistant colonies was determined essentially as described by Horsten and Fehrmann (1980); spore suspensions are diluted as required before plating onto MBC containing agar. Each year, at least 15 billion spores were tested from more than sixty lesions. This procedure is very accurate, but time-consuming. However, testing mass mycelial isolates on MBC containg agar may overestimate the frequency of resistance in field populations since, isolates containg both sensitive and resistant strains, will be recorded only as resistant.

Results and conclusions

During the first five years (1976-80) the frequency of MBC resistant strains increased slowly to 1 in 10^8. Between 1980-82, there was a tremendous increase in the resistant population. In 1982 one in 14 spores was MBC-resistant in samples from 40 lesions from the sprayed part of the field. The frequency in samples from unsprayed plants was one in 10^8.

YEAR	number of tested spores	number of resistant colonies	1 spore resistant out of total
UNTREATED PLOT			
1980	17.300.000.000	96	180.000.000
1982	3.234.000.000	28	120.000.000
TREATED PLOT			
1980	13.440.000.000	137	100.000.000
1982	104.700	about 7.400	14

These results were confirmed by another test procedure based on the effects of MBC on the spore germination. Increases in yield during 1976-80 were considerably higher than in 1981 and 1982. In 1982, control of eyespot was poor.

It is not clear what the practical implications of these findings will be. In our experiments MBC fungicides were applied at the highest rate and frequency possible in W.Germany, where seven years continuous wheat is rather unusual. However, reports from England suggest that failure of MBC to control eyespot may be due to resistance (GRIffin & Yarham 1983).

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3C—R11

PESTICIDE RESISTANCE OF SPIDER MITES IN CZECHOSLOVAKIA

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Background and objectives

Outbreaks of the two-spotted spider mite Tetranychus urticae and the fruit tree red spider mite Panonychus ulmi occur annually in greenhouses, hopgardens, apple orchards and vineyards in Czechoslovakia. These crops have been treated intensively since the fifties with organophosphorus insecticides, and their effectiveness against spider mite populations has gradually declined. At present chemical control has failed completely in some greenhouses due to resistance of T. urticae (and also Myzus persicae and T. vaporarium) and biological methods of control are being introduced.

Materials and Methods

The method recommended by FAO for detection and measurement of resistance in spider mites (Anon. 1969) was modified by spraying leaf discs in a settling tower. Females of sampled populations were exposed on the treated discs. "Leverkusen N", and OP-sensitive strain of T. urticae, and S-strain "Chelčice" of P. ulmi were used as standard susceptible reference strains and resistance factors (RF) are based on comparison of LD50 values.

Results and conclusions

Surveys showed that populations of T. urticae from 35 commercial greenhouses varied widely in their resistance to thiometon (RF = 1.5 - 208.5), but less so to tetradifon (RF = 1.6 - 19.6) in 1974-1976.

In 24 field populations of T. urticae from Bohemian hop-gardens a high level resistance to thiometon was also found. Cross resistance occurred to metidathion, mevinphos, vamidothion and naled, but not to specific acaricides, and cyhexatin, amitraz and dicofol are now applied on half the Bohemian hop-growing area.

Populations of P. ulmi from apple orchards in Bohemia are resistant both to thiometon (RF - 10.5 - 102.8) and to fenitrothion (RF = 8.5 - 68.0). Outbreaks of P. ulmi are widespread but have been kept in check during the last five years with specific acaricides.

Diapause in femals of T. urticae is of substantial importance for the practical management of this secondary pest (Hůrková & Weyda 1982) because of their enhanced tolerance to pesticides. Diapausing females do not affect the host plant, because they neither feed nor reproduce.

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MONITORING FOR INSECTICIDE RESISTANCE IN APHID PESTS OF FIELD CROPS IN ENGLAND AND WALES

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Background and objectives

Since 1980, a programme of insecticide resistance testing has been done at Harpenden to provide a back-up service to Entomologists of the Agricultural Development and Advisory Service. The aims of the programme are three-fold, namely to determine the incidence and distribution of resistant strains in species where resistance is already known to occur, to determine baseline data for species where resistance is, as yet, undetected, including the initial response to newly introduced insecticides, and to monitor such species to try to detect changes in response.

Materials and Methods

Samples of aphids were collected from field crops by Advisory Entomologists, many from situations where failure of control measures indicated the possibility of insecticide resistance. Samples were tested, either on receipt or after culturing, mostly by insecticide bioassay using the cage dip technique (Stribley *et al*, 1983). In the case of peach potato aphid *Myzus persicae*, where insecticide resistance is associated with increased production of Esterase 4, ten aphids per sample were tested individually by electrophoresis (Devonshire, 1975).

Results and conclusions

A total of 145 samples of *M. persicae* were tested, from 1980 to 1982, to determine the incidence and distribution of susceptible and the resistant R1 and R2 field strains. Susceptible aphids only were found in 14 samples, 96 samples contained mixtures of susceptible and R1 aphids and 35 samples contained at least one R2 aphid. In Eastern Region, where the greatest insecticide selection pressure can be expected, moderately resistant R1 aphids occurred in 89 per cent of the samples tested. However, the greatest incidence of strongly resistant R2 aphids occurred in Northern Region (38 per cent of samples tested) where insecticide selection pressure should be less. To determine baselines for the cabbage aphid *Brevicoryne brassicae*, 127 dose-response line tests against demeton-S-methyl were done on 87 single female clonal cultures derived from 33 field populations of the insect. Where possible, repeat tests were done on the same clones to confirm the reproducibility of test results. All clones gave LC50 values within the range of 0.007 to 0.028 per cent active ingredient, indicating only small differences between clones. Field populations are being monitored further to check for responses outside the range so far detected.

To try to detect resistance in the potato aphid *Macrosiphum euphorbiae* and the cereal aphids *Rhopalosiphum padi, Sitobion avenae* and *Metopolophium dirhodum*, single-dose insecticide bioassays were done using previously determined discriminating doses of demeton-S-methyl for *M. euphorbiae* (Anon , 1982) and of demeton-S-methyl and pirimicarb for the cereal aphids (Stribley *et al*, 1983). All 44 samples of *M. euphorbiae* and all 84 samples of cereal aphids tested were killed at the discriminating dose.

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3C—R13

RESISTANCE TO INSECTICIDES IN THE PREDATORY MITE TYPHLODROMUS PYRI AND ITS SPIDER MITE PREY

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Background and objectives

Resistance to organophosphates (OPs) in Panonychus ulmi developed from c. 1960, reaching high levels (Cranham, 1982); the continued use of OPs for insect control has resulted some 20 years later in development of resistance in the predatory phytoseiid mite Typhlodromus pyri. Results are reported of bioassays and field trials to evaluate resistance of the predator to a range of insecticides, and the effects on the balance of predator and prey in orchards.

Materials and methods

T. pyri stocks collected from sprayed and unsprayed orchards were reared separately on plate cultures with pollen of Vicia faba as food, and bioassayed using a taped slide technique (Kapetanakis & Cranham 1983). Insecticides were also evaluated in well-replicated field trials of randomised block design, on young apple trees sprayed to 'point of drip', both mite species being present. Numbers of mites on leaf samples were assessed, using a brushing machine, at successive 3-week intervals after spraying, and when possible before spraying.

Results and conclusions

In bioassays, T. pyri collected from isolated apple trees in North Wales provided standards of 'base-line' susceptibility for carbaryl and nine OPs in common use, as follows. T. pyri was moderately tolerant to carbaryl (LC_{50} 1000 mg/l). With the OPs there was a large ($>$200 fold) range of LC_{50}s in the order phosalone (500 mg/l), fenitrothion, azinphos-methyl, pirimiphos-methyl, chlorpyrifos, parathion, demeton-S-methyl, vamidothion and dimethoate (2.6 mg/l).

Stocks of T. pyri with a long history of exposure to OPs and carbaryl (three native stocks, one from New Zealand) were resistant to these insecticides. The cross-resistance spectrum was similar for all four test stocks: azinphos-methyl, pirimiphos-methyl and dimethoate had low ($<$10) resistance factors whilst the other OPs gave factors in the range 35-76, and carbaryl $>$20. The range of LC_{50}s was again very large, and suggested that carbaryl and certain OPs were probably harmless in practice to resistant T. pyri, whilst others might be harmful. Results of field trials on resistant populations were in agreement; phosalone, fenitrothion, chlorpyrifos, azinphos-methyl and demeton-S-methyl were harmless at normal field rates, and dimethoate and pirimiphos-methyl were very harmful.

Each phytoseiid species that has developed OP resistance exhibits a specific cross-resistance spectrum with a wide range of responses to different OPs; the differences between species in the responses to certain OPs are very pronounced (Croft, 1982). In England, T. pyri provides excellent stable regulation of P. ulmi at non-injurious numbers. A range of OPs, and carbaryl, can now be used as selective agents for insect pest control, without harm to T. pyri. Several non-acaricidal fungicides are available. By a suitable choice of pesticides, resistance in the predator can be exploited, in order to minimise use of acaricides and so prevent further development of resistance in P. ulmi.

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ORGANO-TIN RESISTANCE IN TWO-SPOTTED MITE, *TETRANYCHUS URTICAE* KOCH (ACARINA: TETRANYCHIDAE) IN AUSTRALIA

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Background and objectives

Organo-tin miticides are widely used in Australia. Resistance to cyhexatin was detected in laboratory tests on females of 2 strains of *Tetranychus urticae* from apple and pear orchards in 1981 (Edge and James, 1982). The present investigation was conducted to determine the extent of resistance and the cross resistance pattern of cyhexatin resistant strains, and to identify alternative miticides.

Materials and methods

Samples of *T. urticae* were collected from apple and pear orchards in Victoria and N.S.W. Laboratory cultures were established and acaricides tested against these strains as described by Edge and James (1982).

Results and conclusions

Nine strains of *T. urticae* from 11 orchards in the Goulburn Valley, Victoria, the main pear producing area in Australia, were resistant to cyhexatin. Resistance was also detected in 4 strains of *T. urticae* from 4 orchards in southern N.S.W., but there is no evidence of resistance in mites from the major apple growing areas of the State.

Resistance levels to a wettable powder formulation of cyhexatin 'Plictran 50W' were no more than 8-fold in females of most strains tested. 0.04% cyhexatin usually gave 100% mortality, after 48 h, but with one Victorian strain (ShJ) the dosage/response line departed from linearity at higher concentrations of 'Plictran 50W' and complete kill was not achieved with 0.8% cyhexatin. This effect was less apparent with a flowable formulation of cyhexatin 'Plictran 600F', and was not evident with an emulsifiable concentrate formulation. Tests with cyhexatin, azocyclotin and fenbutatin oxide against females and larvae of this and other strains showed that both stages were resistant to the 3 organo-tin miticides, but resistance levels were lower in larvae.

ShJ was selected in the laboratory with 2 sprays of cyhexatin at the registered rate and 3 sprays at double this rate. Selection increased the LC50 2-fold, but the LC95 was not changed. ShJ was also resistant to organophosphorus compounds, tetradifon and propargite, but susceptible to bromopropylate. A cyhexatin resistant strain was maintained in the laboratory for ca. 70 generations in the absence of any miticide. Resistance to 'Plictran 50W' decreased from 7.5-fold to 2-fold at the LC50 level, but 20% of the population still survived a discriminating dosage of 0.005% cyhexatin, indicating that reversion was unlikely to be a significant factor in the field.

The impact of organo-tin resistance has been greater in pears than apples because of the lower threshold for mite damage in the former. In a replicated field trial against ShJ, 5 sprays of 'Plictran 50W' or 'Plictran 600F', or 4 bromopropylate sprays, applied by knapsack sprayer at the registered rates, prevented economic damage to small pear trees, while 30-50% defoliation occurred in unsprayed plots. The addition of tetradifon to 'Plictran 600F' did not improve control. In 7 commercial blocks of pears in this orchard, 7-8 miticide applications from an air-blast sprayer prevented any defoliation. All blocks were sprayed once with bromopropylate early in the season and once post-harvest with propargite which is phytotoxic to the fruit. Each block also received 0-3 sprays of bromopropylate and 3-5 sprays of cyhexatin in different sequences.

Although organo-tin resistant *T. urticae* can still be controlled in Australian orchards with cyhexatin, the number of applications required in pears has more than doubled since the development of resistance and the cost is becoming prohibitive. Alternation of organo-tins with effective miticides from other chemical groups is considered the most appropriate strategy at present. However, there is a shortage of the latter mainly due to resistance and new miticides are urgently required.

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3C—R15

DEVELOPMENT OF RESISTANCE TO PHOSPHINE BY INSECT PESTS OF STORED GRAINS

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Background and objectives

Phosphine differs from other fumigants in that it is generated *in situ* by decomposition of aluminium or magnesium phosphide. Phosphine is liberated from these phosphides by reaction with atmospheric moisture and is released over a period of two days. Due to its ease of application and effectiveness at low concentrations, phosphine is widely used for treatment of infested grains and other durable agricultural produce in developing countries of the tropics and sub-tropics. Fumigation of grains would be impracticable in many situations if phosphine were not available.

A survey carried out by FAO in 1972/3 showed that some storage pests, in particular *Rhyzopertha dominica* had developed resistance to phosphine. These observations have been confirmed by later studies. It was suspected that development of resistance was due to the repeated use of poor techniques of fumigation. During 1981, it was found that fumigations of grain in Bangladesh were failing despite attempts to up-grade techniques to an acceptable standard. It was decided that a TDRI team should visit Bangladesh to investigate the cause of such fumigation failures.

Materials and methods

The TDRI team carried out five separate experimental fumigations over a period of some four weeks, three at an application rate of 3g phosphine per m^3 and two at 1g phosphine per m^3. Two fumigations were of sheeted stacks of grain while the other three were of bagged grain in warehouses, after sealing gaps and doors, etc. so as to minimise leakages of gas. Concentrations of gas were measured while its effect on insects in cages was assessed.

Results and conclusions

TABLE 1 - Results of experimental fumigations with phosphine applied at 3g m^3

Situation	Concentration-time product (mg.hr/litre)			Insects surviving fumigation					
				Exposed in cages within stacks		Sieved from grain samples from stacks		Collected from papers laid on floors	
	24hr	72hr	96hr	A	B	B		B	
Improved Dacca warehouse	-	43	45	0	Rd	So Cr	Rd	Rd	Cr
Modern warehouse	-	67	79	0	Cr	Rd			Cr
Sheeted stack	-	175	238	0	0	0			Nt

Nt - Not tested; B - Bangladesh strain; A - Susceptible laboratory strain;
So - *Sitophilus oryzae*; Cr - *Cryptolestes* species; Rd - *Rhyzopertha dominica*.

It may be concluded from Table 1 that considerable resistance to phosphine has developed in Bangladesh, particularly by *R. dominica*. This has been caused by persistent fumigation of bagged grain without ensuring that adequate concentration-time products of phosphine were achieved to ensure effective control of all species of pest. Gas-tight sheets should be used to cover bag stacks and adequate dosages of phosphine should be applied.

Development of resistance to phosphine by insect pests would severely limit its use as a fumigant with serious consequences both for world trade and the prevention of food losses in developing countries. A programme is needed to ensure that all fumigations are carried out properly and, where possible and desirable, methyl bromide should be used as an alternative to phosphine.

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THE DISPERSIVE EFFECTS OF SOME INSECTICIDES ON RESISTANT APHIDS; IMPLICATIONS FOR VIRUS DISEASE CONTROL

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Background and objectives

Good cultural practices and the use of disease-resistant crop varieties are important for virus control but must be supplemented by use of insecticides to restrict the spread of some aphid-borne viruses. Development of insecticide resistance in *Myzus persicae* (Sulz.) has, by prolonging survival, extended sub-lethal exposure and increased the risk that insecticide-induced dispersal might cause virus spread.

Our experiments investigated the effects of some insecticides on aphid movement and transfer of beet yellows virus (BYV) and potato virus Y(PVY).

Materials and Methods

Apterous colonies of *M. persicae* susceptible (S), moderately (R1) or strongly (R2) resistant to most insecticides were established on the undersides of leaves infected with BYV or PVY. Leaves were sprayed or brushed with the organophosphorus insecticide demeton-S-methyl (500mg ai litre^{-1}), the carbamate pirimicarb (300mg ai litre^{-1}) or the pyrethroid deltamethrin (10mg ai litre^{-1}). Aphid movements were monitored by time-lapse videotape recording and dispersal quantified by counting aphids which moved more than 120mm. Dispersed aphids were transferred to indicator seedlings of *Beta vulgaris* L. cv. Hilleshog monotri or *Nicotiana tabacum* L. cv. White Burley, to assess their abililty to transmit BYV or PVY, respectively.

Results and conclusions

Demeton-S-methyl or pirimicarb caused aphids to secrete alarm pheromone, released from droplets produced on their cornicles. The pheromone from one individual caused nearby aphids to disperse and, as more aphids secreted in response to ingesting the insecticide, the whole colony dispersed. The timing and extent of the dispersive response was influenced by aphid population structure, density and level of resistance. Transmission of viruses to indicator seedlings depended on dispersed aphids surviving long enough, i.e. several hours for the semipersistent BYV but only a few minutes for the non-persistent PVY. Therefore, insecticide-induced spread of PVY is more likely than of BYV although, in our tests, BYV was spread when dispersal was rapid and the aphids were strongly insecticide-resistant.

Treatment with deltamethrin did not induce cornicle secretion but caused some dispersal by inducing hyperactivity which was, however, rapidly followed by incapacitation so that little virus was transferred. Deltamethrin, a non-systemic insecticide, applied to field crops might not reach aphids underneath large leaves but if such aphids moved on to treated foliage they would be likely to acquire an immobilising dose of this fast-acting pyrethroid. Because deltamethrin can restrict movement it might, in mixtures, counter the aphid-dispersive effects of demeton-S-methyl or pirimicarb, thus optimising aphicidal activity whilst minimising virus spread.

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3C—R17

VARIATION IN RHIZOCTONIA CEREALIS TO FUNGICIDES

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Background and objectives

The incidence of sharp eyespot infection, caused by *Rhizoctonia cerealis* (Boerema & Verhoeven, 1977), in some cereal crops has been reported to be increased following treatment with benzimidazole fungicides (e.g. Hoeven & Bollen, 1980). The response is variable, however, and more information is required of the sensitivity of the pathogen to these chemicals. The aim of the present study was to compare the reaction *in vitro* of isolates of *R. cerealis* obtained from different cereals to carbendazim, benomyl and thiophanate-methyl.

Materials and Methods

Isolates of *R. cerealis*, obtained from plants infected with sharp eyespot in crops of wheat (W) barley (B) and oats (O) in the United Kingdom and crops of rye (R) in Holland, were grown on replicate plates of potato-dextrose agar supplemented with the fungicides carbendazim ('Bavistin', 50% a.i. w.p.) benomyl ('Benlate', 50% a.i. w.p.) and thiophanate-methyl ('Cercobin', 50% a.i. liquid) at 22°C in the dark. Mycelial growth was measured after 5 d.

Results and conclusions

TABLE

Sensitivity of *R. cerealis* to fungicides

Isolate	Host	LD50 of mycelial growth (µg a.i./ml)		
		carbendazim	benomyl	thiophanate-methyl
82/1	W	7	5	60
82/4	W	6	3	58
82/211	W	10	8.5	78
79/74	W	10	9	120
79/59	W	12	10	205
79/5	B	16	10	55
79/29	O	18.5	9	95
78/62	R	15	12	160
76/482	R	9	7	190
75/376	R	12	8	3000

Mycelial growth of all of the isolates was inhibited most strongly by benomyl and the extremes of sensitivity differed by fourfold. Growth was not stimulated *in vitro* by low concentrations of either benomyl or carbendazim. These results support the suggestion that the stimulation of sharp eyespot following treatment with benomyl or carbendazim may be due to indirect effects of the fungicides on antagonists of the pathogen (Hoeven & Bollen, 1980). In contrast, *R. cerealis* was much less sensitive *in vitro* to thiophanate-methyl, and some isolates were directly stimulated by this fungicide at concentrations up to 5 µg a.i./ml. It is not known whether, or to what extent, sharp eyespot might be directly stimulated by this material in the field.

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PERFORMANCE OF BARLEY MILDEW FUNGICIDES IN SOUTH-EAST SCOTLAND

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Background

Spring barley is the main cereal in south-east Scotland. Golden Promise, the predominant cultivar, is extremely susceptible to mildew *(Erysiphe graminis)* which is controlled by the routine use of fungicide seed treatments and sprays. The recent introduction of winter barley, which now accounts for some 10% of the barley area, has increased the risk of early mildew infection and increased fungicide usage. To reduce the carry-over of mildew, winter barley growers are advised to spray infected crops before nearby spring crops braird. In this situation the College also recommends the use of a mildew seed treatment on Golden Promise and other susceptible spring cultivars. Follow-up sprays to control later mildew development in the spring crop are routinely recommended. To reduce the risk of resistance developing in the mildew population, barley growers are advised, whenever possible, to use unrelated fungicides for these three purposes.

Fungicide Trials

Triadimefon has for several years been the most widely used spray against barley mildew in south-east Scotland. Tridemorph also gave good disease control, but was superseded largely because of its noticeably shorter persistence. In recent trials with Golden Promise the relative performance of these fungicides has changed. In 1980 single and double sprays of triadimefon gave better mildew control and outyielded corresponding programmes with tridemorph. As expected the advantage of a two-spray programme over a single spray was less for the more persistent triadimefon. In similar trials in 1981 triadimefon was outyielded by tridemorph programme for programme, and showed the larger advantage of a two-spray programme over single sprays. In 1981 mildew levels were higher and the change in the pattern of yields was associated with poorer mildew control with triadimefon, especially where mildew was allowed to become well established before the fungicides were applied: tridemorph had the superior eradicant effect.

Survey of Mildew Fungicide Use on Spring Barley in 1982

Replies to a postal survey were received from 950 farms in south-east Scotland, giving information on 2,220 combinations of cultivar and fungicide programme, covering almost 70,000 hectares. Overall, 36% of the spring barley was grown from seed treated with a mildew fungicide; 95% was sprayed at least once to control mildew; 39% was sprayed twice; and 2% was sprayed three times.

Golden Promise occupied 76% of the spring barley area. Mildew seed treatments were used on 44% of the Golden Promise. Almost all of this cultivar was sprayed at least once, but mildew seed treatments reduced the need for a second spray. Where a seed treatment was not used 90% of the Golden Promise was first sprayed against mildew with the herbicide. At that time mildew was already well established in many crops and this may have contributed to the relatively poorer performance of those fungicides without a marked eradicant effect. Ethirimol and triadimenol seed treatments were especially effective in reducing the need for the first mildew spray to be applied with the herbicide.

Where mildew seed treatments were not used on Golden Promise, triadimefon constituted 46% of the first mildew sprays, but only 4% of the second sprays. This undoubtedly reflected growers' experience of poor mildew control with this fungicide and specific College advice to switch to an eradicant product in such circumstances. Growers indicated "poor" or "very poor" control on nearly half the area first sprayed with triadimefon or propiconazole, with only one quarter "good" or "excellent". Only 6% of the area first sprayed with fenpropimorph or tridemorph was rated "poor" or "very poor" for control, with 79% "good" or "excellent". Poor control was primarily a problem on the highly susceptible cultivar Golden Promise: all fungicides were given better ratings on the more resistant cultivars. The performance of triadimefon and propiconazole first sprays was also considered better where a mildew seed treatment had been used, with the notable exception of triadimefon following triforine seed treatment. Growers' ratings for fenpropimorph were little affected by seed treatment, but those for tridemorph were poorer where a mildew seed treatment had been used.

Fungicide Insensitivity

Although no systematic survey has been done, several small scale investigations have failed to show any general differences in sensitivity of mildew to triadimenol even where poor control has occurred with this fungicide or with triadimefon.

3C—R19

FIELD EVALUATION OF FUNGICIDE STRATEGIES TO CONTROL BARLEY POWDERY MILDEW

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Background and objectives

Greater use of fungicides to control barley powdery mildew (*Erysiphe graminis* f.sp *hordei*) has contributed to increased yields. More autumn-sown barley, and the introduction of more persistent triazole fungicides extended the period to which mildew is subjected to chemical control. This has generated greater selection for less sensitive pathogen forms. To avoid resistance, growers have been advised to follow strategies involving alternating fungicides with different modes of action. As little experimental evidence exists on which to evaluate these strategies, we examined in 1981-2 the effect of different strategies on the performance of triazole fungicides.

Materials and Methods

A plot (6 x 8m) of spring-sown barley (cv Golden Promise) sandwiched between two similar sized plots of autumn-sown barley (cv Maris Otter) formed the unit to which strategies were applied. Strategies (Table) used triadimenol ("Baytan", formulated with the mbc fungicide fuberidazole), propiconazole ('Tilt') or fenpropimorph ('Corbel'), all at recommended rates. Each fungicide inhibits sterol biosynthesis, but fenpropimorph acts at a different step to the others. Cross-sensitivity between fenpropimorph and the two triazole fungicides has not generally been observed. Each strategy was evaluated on at least two replicate units. Sprays were applied to Maris Otter barley when inoculum was moving to adjacent spring-sown seedlings, and, fungicide sensitivity, disease levels, and yields of spring barley were used to evaluate each strategy. Sensitivity of mildew from Golden Promise to triadimenol was determined in the laboratory as described by Hollomon & Butters (1981).

Results and conclusions

Alternating triazole fungicides with fenpropimorph controlled mildew and increased yields of Golden Promise (Table). Disease control was less satisfactory where only triazole fungicides were used and yields of spring barley actually diminished. These differences in performance could not be related to triadimenol sensitivity. Sequential use of three triazole treatments did not reduce the sensitivity of mildew to triadimenol, when compared to its sensitivity in plots receiving no fungicide. Mildew in untreated plots was, however, less sensitive to triadimenol in 1982 than in 1981 (Hollomon & Butters 1981) suggesting that changes had occurred in the natural population in that time.

Differences between strategies seemed related to effects of fungicides in disease spread rather than to changes in fungicide sensitivity. Fenpropimorph controlled mildew more effectively than propiconazole, and initial inoculum levels reaching adjacent Golden Promise were lower. This, together with the effect of triadimenol in reducing the rate of disease spread (r), combined to slow down mildew development in those strategies where triadimenol followed fenpropimorph. If propiconazole was used instead, more inoculum entered spring-sown plots, disease spread more rapidly and foliage protected during the early stages of crop growth became heavily infected at ear emergence. This perhaps contributed to lower yields in strategies using only triazoles.

TABLE Evaluation of fungicide strategies to control barley powdery mildew

Strategies: Autumn sown Seed treatment	Strategies: Spray	Strategies: Spring sown Seed treatment	Mildew infection on Golden Promise % leaf area infected GS 24	GS 39	GS 45	Triadimenol Sensitivity ED50 ug/ml	Yield of Golden Promise t/ha
NONE	NONE	NONE	40	51	34	0.031	5.87
T	P	T	3	19	26	0.037	5.17
T	F	T	1	11	8	0.029	6.33
	LSD 5%		10	14	15	0.010	0.43

T = Triadimenol; P = Propiconazole; F = Fenpropimorph

Reference

Hollomon, D.W.; Butters, J.A. (1981) Impact of fungicide treatment of winter barley on disease control in spring barley. Proceedings 1981 British Crop Protection Conference - Pests and Diseases 651-657.

FUNGICIDE INSENSITIVITY AND HOST PATHOGENICITY IN BARLEY MILDEW

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Background and objectives

Effective and widely used disease control agents select the controlled pathogen for an appropriate response. The rapid increase in use of triazole fungicides for the control of cereal diseases was therefore expected to select for a response, particularly in *Erysiphe graminis* f. sp. *hordei* (powdery mildew), which adapts more rapidly to control measures than other pathogens. It was also possible that selection for fungicide insensitivity in the pathogen might depend on the particular variety on which it was occurring.

Materials and Methods

The pathogen population was monitored by exposing seedlings of a susceptible variety, Golden Promise, grown from untreated seed, or from seed treated at 0.025 or 0.075g^{-1} a.i. triadimenol per kg^{-1} seed, in a wind impaction spore trap (WIST) mounted on a car roof. WIST exposures were made regularly on a 200km circuit south and east of Cambridge, and on a transect from Cambridge to the north of Scotland. Insensitivity was assessed by comparing the numbers of colonies incubated on treated seedlings relative to those on untreated.

The field trial compared varieties with specific resistance genes either untreated or treated at the commercial rate with ethirimol or triadimenol. Each individual plot (approx. 4m x 1.5m) was surrounded by a 1.5m guard of spring rye to limit interference between plots.

Results and Conclusions

East and south of Cambridge there was an increase in fungicide insensitivity. Early in 1981, the number of colonies on treated seedlings (0.025g^{-1} a.i. treatment) was about 12% of that on untreated seedlings, rising to about 70% early in 1983. Early in 1982, the numbers of colonies on seedlings with the higher fungicide treatment (0.075 g^{-1} a.i.) was about 15% rising to more than 30% early in 1983. In Scotland and northern England, a similar trend occurred earlier (Fletcher & Wolfe, 1981), but reversed slightly in 1982. This may have been due to changes in fungicide use in the north in 1982, causing less selection for insensitivity to triazole fungicides. Also, the lesser relative importance of triazole-treated winter barley may have led to decreased survival of fungicide insensitive genotypes in the north. Differences between the increases in insensitivity at the two rates of treatment indicated that more than one mechanism of insensitivity may have been involved.

Insensitivity to triazoles was found to be associated with pathogenicity for varieties with the *Mla6* resistance gene, similar to the association between insensitivity to ethirimol and pathogenicity for varieties with the *Mla12* resistance gene (Wolfe & Dinoor, 1973). These interactions were tested in a field trial (Table 1).

TABLE 1. Per cent mildew infection (GS 45) and yield (t/ha^{-1}) of barley varieties untreated, or treated with ethirimol or triadimenol (lsd, $P < 0.05$: 0.40 for infection, 0.47 for yield).

Variety	Mildew infection 0	ethir.	triad.	Yield 0	ethir.	triad.
Golden Promise	36.0	20.3	20.3	4.46	5.33	5.19
Midas (*Mla6*)	19.2	2.9	6.4	5.71	6.02	6.66
Carnival (*Mla6*)	5.4	0	0.6	7.95	8.68	8.34
Egmont (*Mla12*)	18.1	10.6	0.8	7.41	8.12	9.24
Hassan (*Mla12*)	20.3	13.3	6.1	5.85	5.86	6.78

From Table 1, ethirimol was more effective than triadimenol in controlling mildew on the *Mla6* varieties, but the converse was true on the *Mla12* varieties. This pattern was reflected in the yield data which suggest that for the *Mla6* varieties, triadimenol treatment was less cost-effective than for the *Mla12* varieties, and that for the *Mla12* varieties ethirimol was less cost-effective than triadimenol. These effects were related to the local occurrence of fungicide-insensitive genotypes of the pathogen.

Continued intensive use of the triazoles is likely to lead a further increase in pathogen insensitivity and loss of fungicide effectiveness. This may be partially offset by avoiding treatment of varieties with *Mla6* resistance, and by concentration on other strategies of fungicide and varietal diversification that will delay the rate of pathogen response.

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✓ **3C–R21**

SELECTIVE MODE OF ACTION OF SIMETRYN IN GRAMINEOUS PLANTS

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Background and objectives

Simetryn (2,4-bis(ethylamino)-6-methylthio-1,3,5-triazine) is a selective herbicide which controls broadleaf weeds and barnyardgrass in paddy rice. The mechanism of this selectivity between different grasses is not fully understood, but selectivity is expressed from both root and shoot applications. To clarify the mechanism of the selectivity, the absorption, translocation and metabolism of simetryn, and its site of action were investigated in rice (*Oryza sativa* L. cv. Nihonbare) and barnyardgrass (*Echinochloa oryzicola* Vasing.).

Materials and Methods

Rice, barnyardgrass, corn (*Zea mays* L.), wheat(*Triticum aestivum* L.), large crabgrass (*Digitaria adscendens* Henr.) and finger millet (*Eleusine coracana* Gaertn.) were selected and their response to simetryn were investigated when applied to shoots and roots. Plants were grown in a controlled growth chamber to the 3-leaf stage in water culture. Foliar and root application were done by dipping the shoots and roots in a simetryn solution at various concentrations, respectively, for one hour.

14C-labeled simetryn was supplied to roots and shoots, and rates of absorption and translocation determined by combustion of a sample together with autoradiography. Metabolism was investigated by thin-layer chromatography. Photochemically active chloroplasts were isolated from plants, and the effect of simetryn on the photochemical reactions monitored with an oxygen electrode.

Results and conclusion

Rice showed tolerance to foliar-applied simetryn, and rice and corn showed it to root-applied simetryn. Barnyardgrass, large crabgrass and finger millet showed susceptibility at both applications. Rice and barnyardgrass were chosen for further studies because both species grow competitively in the same ecological environment and were different in their response to simetryn.

From foliar applications, the rate of absorption of 14C-simetryn was much greater in barnyardgrass than in rice, but few differences in translocation between the two species were observed. No significant differences in the rate of degradation of 14C-simetryn were detected, but the main pathways of metabolic degradation were remarkably different in each species. Rice converted simetryn to water-soluble metabolites and methanol-insoluble residues, whereas in barnyardgrass it was mainly to the mono-dealkylated derivertive. It is concluded that selectivity from foliar applications is mainly due to differences in the rates of absorption and metabolic activity in shoots.

From root applications, the rates of absorption were almost identical in both species, but the rate of translocation from roots to shoots was greater in barnyardgrass than in rice. In roots, 14C-simetryn was also metabolized to water-soluble metabolites and insoluble residues as well as other dicloromethane-soluble metabolites , but metabolic activity was much greater in rice. At 24 hours after treatment, little amounts of unchanged simetryn was detected in roots of rice. Concentration of unchanged simetryn in shoots was remarkably different. At 24 hours after treatment, 13.7 % and 31.0 % of total radioactivity in shoots was identified as unchanged simetryn in rice and barnyardgrass, respectively. It is concluded that the selectivity of simetryn when applied to roots is mainly due to differences in metabolic activity in both roots and shoots, and in the rate of translocation from roots to shoots.

Simetryn severely inhibited electron transport of photosystem II in both chloroplasts (I50 concentration was about 5×10^{-8} M). Mono-dealkylated simetryn was also inhibitory (I50 concentration of photosystem II was about 10^{-6} M), but other simetryn analogues were non-toxic. No inhibition of photosystem I dependent electron transport was observed up to 10^{-4} M. Non-cyclic photophosphorylation was inhibited at the same concentration as photosystem II. However, chloroplasts from both rice and barnyardgrass were equally sensitive in all the photochemical reactions tested. Differences at the site of action, therefore, do not appear to contribute to the selectivity of simetryn between rice and barnyardgrass.

RESISTANCE OF THE EGYPTIAN COTTON LEAFWORM TO SYNTHETIC PYRETHROID INSECTICIDES

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Background and objectives

Synthetic pyrethroids are used to control cotton pests in Egypt. Resistance has developed in the Egyptian cotton leafworm, *Spodoptera littoralis*, to certain insecticides. The aims of this study were to develop strains of *S. littoralis* resistant to pyrethroids, study their cross resistance, and clarify the relationships between resistance and certain enzymatic activities.

Materials and Methods

4th instar larvae of a field strain of *S. littoralis* were topically treated with technical cypermethrin and deltamethrin dissolved in acetone. Mortality was recorded 24h later. LD25 was used to select 5000 larvae (4th instar) and survivors reared to the first generation (F1). Selection was continued on progenies in this way until the 12th generation (F12). A relatively susceptible (S) strain, reared without treatment under the same conditions was used as a standard. After F12, cross resistance of 4th instar larvae of cypermethrin-resistant (CR) and deltamethrin-resistant (DR) strains was measured. Activities of AchE, AliE[1], a and bE[2], AcPase and AlkPase[3] were determined in the 6th instar larvae of F12 of both resistant and susceptible strains.

Results and conclusions

Resistance in CR did not increase after selecting with the LD25 for four generations, while DR reached the same limit after six generations. There was, in general, cross resistance in CR and DR to the two insecticides, and a possible correlation with chlorinated hydrocarbon or organophosphate-resistance (Table 1).

TABLE 1

Cross resistance of CR and DR strains to different insecticides

INSECTICIDES	CR*	DR	INSECTICIDES	CR	DR
Cypermethrin	27.0	11.5	Methomyl	0.7	1.0
Deltamethrin	3.5	17.0	Endrin	5.3	5.1
Fenpropathrin	0.9	4.9	Lepicron	2.7	3.7
Fenvalerate	2.5	11.3	Chlorpyriphos	3.4	4.0
			Fenitrothion	2.5	5.8
			Profenophos	1.0	1.6

*All figures are resistance factors. LD50 of S strain was 0.038 and 0.009 µg/g body weight for Cypermethrin and Deltamethrin respectively.

Certain hydrolase activities are shown in Table 2. CR and DR had higher activities of AchE, AliE and non specific esterases (a & b) than the susceptible strain whereas AlkPase activity was lower. These results indicate that resistance of *S. littoralis* to synthetic pyrethroids might be associated with the activity of such enzymes as AchE, AliE and a & b-E.

TABLE 2

Hydrolytic enzyme activities of CR, DR and S strains of *S. littoralis*

	Activity x 10-3 of					
Strains	AchE(1)	AliE(1)	a-E(2)	b-E(2)	AcPase(3)	AlkPase(3)
CR	302	238	2062	444	15.9	2.42
DR	295	315	2472	454	16.2	4.30
S	111	161	873	190	17.7	6.11

Figures are means of 3 replicate determinations each based on three insects.
1. mg AchBr and Meb hydrolyzed /mg.protein/30 min. 2. µg a and b naphthol released /mg.protein/30 min. 3. mg phenol released /mg.protein/60 min.

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3C—R23

MECHANISM OF ACARICIDE RESISTANCE IN CITRUS RED MITE WITH EMPHASIS ON BENZOXIMATE

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Background and objectives

Development of resistance in mites to acaricides is a worldwide problem. Resistance can develop rapidly to many compounds and reach very high levels. Studies of mechanisms of resistance to acaricides in mites have lagged behind comparable works in insects, because of handling difficulties presented by their minute size. It has been studied with dicofol (Saito et al. 1983) and some organophosphorus compounds. Benzoximate (ethyl O-benzoyl 3-chloro-2,6-dimethoxybenzohydroximate) showed high activity against citrus red mite and was first used 1971 in Japan. However benzoximate resistant mites appeared after 2 years. The following experiments were undertaken to study the mechanism of resistance as this might provide information that would help counteract the resistanceproblem.

Materials and Methods

The toxicity of benzoximate to female adults of citrus red mite of susceptible (Shizuoka S and Fukuoka S) and resistant (Shizuoka R, Fukuoka R and Okitsu R) strains was evaluated by the leaf disc method (Yamada et al. 1983) and slide dip method (Voss 1961). ^{3}H-benzoximate (1.09 mCi/mmol) was synthesized from ^{3}H-dimethylsulfate and 3-chloro-2,6-dihydroxynenzoic acid through four reaction steps. Female adults were dipped in 200 ppm solution of ^{3}H-benzoximate for 5 seconds. Cuticle penetration was examined by washing mites with toluene scintillator. Treated female adults were crushed on silica gel tlc plate and metabolism determined by tlc separation.

Results and conclusions

Twenty-four h after treatment, 17, 41, 49, 60 and 66% of the applied dose had penetrated the cuticle of Okitsu (R), Shizuoka (R), Fukuoka (R), Shizuoka (S) and Fukuoka (S) strains, respectively. Sixty to 75% of applied ^{3}H-benzoximate was recovered unaltered from each strain. The main metabolite was ethy-3-chloro-2,6-dimethoxynenzohydroxamate. It was suggested that metabolism of benzoximate might not be an important factor of benzoximate resistance in citrus red mite and reduced permeability of benzoximate through the cuticle is one mechanism of benzoximate resistance.

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DIAMONDBACK MOTH RESISTANCE TO INSECTICIDES

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Background and objectives

The diamondback moth, *Plutella xylostella*, has long been recognized as a cosmopolitan pest of cruciferous crops. Control currently depends on the extensive use of insecticides which might eventually lead to the development of resistance. In a field survey in 1980 in Taiwan, the diamondback moth was resistant to 15 insecticides from all classes. The present study investigates the biochemical basis for this resistance, and examines the involvement of insensitive acetylcholinesterases in organophosphorus and carbamate resistance, and that of mixed-function oxidases and hydrolases in synthetic pyrethroid resistance.

Materials and methods

Susceptible and locally-collected resistant strains of the diamondback moth were used. The resistance factors for these two strains were reported by Liu *et al*. (1982). Fourth instars were sprayed with acetone solution of the insecticide and mortality was recorded 24 hr later. Synergists were applied either before insecticide treatment at maximal sublethal dosages or together with the insecticides at varying ratios. Inhibition of acetylcholinesterases was determined as described by Hart and O'Brien (1973). For knockdown assay, adults were exposed to the insecticides with or without synergists coated on glass vials. Three temperatures were used to determine the posttreatment temperature effect on the toxicity and knockdown action of synthetic pyrethroids against both susceptible and resistant strains.

Results and conclusions

The mixed-function oxidases inhibitor, piperonyl butoxide, significantly synergized the effects of carbamate insecticides on the resistant strain. This did not, however, account fully for the high levels of resistance to carbamate insecticides. Acetylcholinesterase from the resistant strain was up to 50-fold less sensitive than that from susceptible strain to several organophosphorus and carbamate compounds. This insensitivity was mainly attributable to a decreased affinity of acetylcholinesterase for these insecticides.

Esterase hydrolysis was a factor in permethrin resistance. Oxidation mediated by mixed-function oxidases was an important mechanism for resistance to four synthetic pyrethroids. A substantially longer time was required for synthetic pyrethroids to produce knockdown effects in resistant than in susceptible adult moths. This difference and the temperature effect will be discussed in terms of the involvement of both metabolic and non-metabolic mechanisms in synthetic pyrethroid resistance.

References

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EXCLUSION AS A STRATEGY FOR COMBATING RESISTANT SAW-TOOTHED GRAIN BEETLE

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Background and objectives

In 1973 a survey indicated that strains of *Oryzaephilus surinamensis* resistant to organophosphorus compounds were not present in the U.K. though they occurred in at least ten overseas countries. It was important to exclude such strains from U.K. grain-growing farms where this beetle is the most important pest of stored grain. From 1974 all strains of *O. surinamensis* detected on imports were tested for resistance, and when found infestations were eliminated (e.g. by fumigation). Concurrently the resistance status of inland infestations including those on farms was monitored.

Materials and Methods

Resistance was measured using the filter paper technique recommended by FAO (Anon 1970).

Results and discussion

TABLE 1

Resistance to organophosphorus compounds in *Oryzaephilus surinamensis* 1974-1982

Year	Number of samples tested			Number of samples resistant		
	Imports	Inland except farms	Inland farms	Imports	Inland except farms	Inland farms
1974	49	83	67	9	1	0
1975	71	92	71	12	4	1
1976	41	111	82	18	2	0
1977	36	173	100	18	6	2
1978	28	81	93	8	6	0
1979	32	90	61	12	7	0
1980	22	57	66	8	3	0
1981	23	109	70	7	9	1
1982	11	60	44	5	10	4

Overall the incidence of resistance on imports has been 31%. Inland it has been 1.2% on farms and 5.6% elsewhere, or before 1982 0.7% on farms and 4.8% elsewhere. Many of the 48 resistant strains detected away from farms inland were associated with imported foods, twenty being on dried fruit, rice, nuts, or carobs. It was not anticipated that every imported resistant strain would be detected, but while a high number of import interceptions was maintained (mean 44 per year before 1980) few farm outbreaks of resistance occurred. The rate of import interception dropped in 1980 and this was followed by a farm occurrence in December 1981 and four more in 1982. Resistant strains have now been detected in an intervention store, and in grain submitted for phytosanitary certification before export. They are known to have overwintered in the U.K. and are now probably established here.

Acknowledgement

Much of this work has been undertaken by Miss D.G. Blackman and staff of the Regional Pests Service and DAFS.

Reference

Anon (1970) Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. FAO Plant Protection Bulletin 18, 107-113.

A STRATEGY FOR METALAXYL USE AGAINST BLACK POD OF COCOA

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Background and objectives

Black pod of cocoa, caused by *Phytophthora palmivora* (Butl.) Butl. is the most serious disease of cocoa in Papua New Guinea. Pod losses at Keravat have averaged 25% (range 16-40%) since 1976. All Amazonian x Trinitario hybrids currently available to growers are highly susceptible to the disease and fungicide sprays will almost invariably be required to obtain the high yields expected of the new planting material. Both metalaxyl and cuprous oxide effectively and profitably control black pod on high yielding trees under plantation management conditions.

Metalaxyl sensitivity tests conducted on local isolates in V8 agar culture found the E.C. 50 to be between 0.3 and 0.4 µg/ml. None of the 260 isolates collected from surveys of Ridomil sprayed blocks grew at 1 µg/ml metalaxyl. Even though metalaxyl resistance has not been found in *P. palmivora* on cocoa, reports of resistance in pathogens of annual crops prompted further study of the two fungicides with a view to developing a metalaxyl resistance avoidance strategy. Cuprous oxide, metalaxyl or tank mixtures of the two, were compared using small scale screening methods (McGregor, 1982) to examine relative effectiveness and persistence. Techniques of controlled spraying and washing of detached pods were subsequently developed to test the effectiveness of each fungicide under different ambient conditions with various drying times.

Materials and methods

Trees of susceptible clones were field sprayed by mistblower with metalaxyl (0.35%), cuprous oxide (8% Cu^{++}) or mixtures in the ratio 40:1, 20:1 or 10:1, Cu^{++}: metalaxyl. Detached pods were inoculated with drops of zoospore suspension at intervals after spraying.

Pods of Trinitario clone KA2-101 were also sprayed by mistblower under standardised conditions in the laboratory with 0.4% metalaxyl or with 8% Cu^{++} on hot sunny days, overcast days, or sunny days with half the pods placed in a humid chamber. A standardised washing treatment was applied to each pod to remove non-adhered fungicide deposit at intervals after spraying. Pods were inoculated as above.

Results and conclusions

The proportion of inoculum drops which developed typical spreading lesions was determined five days after inoculation. All field spray treatments were about equally effective and persistent, except when rain fell within two hours of spraying on an overcast day. In this case cuprous oxide was ineffective but metalaxyl was fully effective even though applied within minutes of rainfall. Mixtures were intermediate between the two.

In laboratory tests metalaxyl was fully effective within 24 min of spraying whilst cuprous oxide took 1½ h to dry at low humidity and 2 h at high humidity. Longer drying times improved the effectiveness of the cuprous oxide spray.

Rainfall is unpredictable during the wettest part of the year when black pod is most severe and cuprous oxide may be less effective in commercial practice than metalaxyl. Nevertheless the disease control strategy should rely on cuprous oxide for the less wet parts of the year when good spraying days are more frequent. Growers should change over to metalaxyl when the wet season begins and continue until the weather becomes more predictable utilising the valuable rain-proof properties of metalaxyl at a critical period in the black pod epidemic. This critical period variation of the alternating strategy should permit optimal use of the two fungicides until other highly effective systemic fungicides with a different mode of action are available for use as mixing partners with metalaxyl.

Reference

McGregor, A.J. (1982) A small-scale screening technique for evaluating fungicides against *Phytophthora palmivora* pod rot of cocoa. *Annals of Applied Biology*, 101, 25-31.