THE PROBLEM OF FUNGICIDE RESISTANCE OF PLANT PATHOGENIC FUNGI

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Abstract

Schlosser, E. 1986. The problem of fungicide resistance of plant pathogenic fungi. Arab J. of Pl. Prot. 4: 124 – 120

Development of fungicide resistance of plant pathogenic fungi is a world-wide Phenomenon. Based on selected examples, factors responsible for resistance development are presented. Means of overcoming already existing resistance problems are discussed as well as strategies to prevent or retard a build-up of fungicide resistant fungal populations.

Additional key words: fungi, fungicide resistance.

As far as I am aware, The Arab Countries do not use fungicides to a large extent. Thus, there are no immediate problems of fungicide resistance – yet. Populations in the Arab World are steadily increasing, creating the need for more food. This goal can only be achieved by a more intensified agricultural production, which almost invariably leads to higher application rates of pesticides in general, including fungicides. My topic may appear premature or even exotic at this congress – but sooner or later, the Arab Countries will have to face the same problems as we have to do right now. This means, that they are at present in a better position as we are in Europe or elsewhere. They can still act and try to prevent future troubles, while we have mostly no other choice as to react to already existing problems. From the various aspects of fungicide I will deal with the following topics:

- characterization of resistant fungal strains
- factors determining resistance development.
- strategies to prevent or retard resistance.

It will not be possible to cover the whole field on this occasion. Thus, my presentation is nothing more but an attempt to channel future activities in plant protection in this part of the world into a certain direction.

Characterization of Resistant Fungal Strains

The failure of a fungicide to control a target organism is not necessarily conclusive evidence for the presence of resistant fungal strains (RFS). For hitherto unknown reasons, such failures do occur even in carefully designed and executed field trials. In such an event, only laboratory tests can prove the existance of RFS. Once they have been demonstrated, various parameters should be checked in order to understand their meaning under field conditions.

Regional Profile: Comprehensive samples should be collected in representative fields from all areas in which the respective crop is grown. A laboratory analysis will reveal the precentage of RFS within the fungal populations. It will further inform whether the occurence of RFS is still a localized event or is widespread.

Tolerance Level: Once the presence of RFS has been established the tolerance of the pathogen to a given fungicide has to be determined. This usually done by dosis-effect studies with concentration gradients in or on a substrate, which is either nutrient agar or plant material.

Definition of Resistance: Sensitive strains (SFS) within fungal populations vary considerably in their tolerance to a fungicide. A 2 – to 5 – fold higher ED_{100} is certainly within the range of natural variation. At which concentration may we consider a pathogen to be resistant? This is a matter of definition, which is of course arbitrary. In our studies a pathogen is considered resistant, when it tolerates at least a 10 – fold dose of a fungicide without any apparent effect on mycelial growth and sporulation in or on a substrate.

Type of Resistance: An important step in the characterization of RFS is the determination, whether resistance is adaptive or constitutive. The first is only transitory, while the second is permanent. This question can be answered by 5– 7 passage of the pathogen on a fungicide – free substrate with subsquent check of its growth on a substrate supplemented with the fungicide (Figure 1). When resistance is still pronounced and is comparable to that of the first isolation, it may be considered constitutive. When it has been lost during the passage, as indicated in the lower part of Figure 1, the resistance has only been adaptive. This difference is quite important for a critical evaluation and the consequences of a resistance situation.

Cross Resistance: By definition, this term implies that a pathogen resistant to a fungicide will exhibit likewise resistance to all other related fungicides. This effect is not always linked to similar chemical structures of the active ingredients, but is rather dependent on a same mode of action. As an example, *Sphaerotheca fuliginea* the causal agent of powdery mildew on cucurbits, is resistant to triadimefon and triforine as well despite their completely unrelated chemical structures. Both are ergosterol – biosynthesis – inhibitors (EBI) with the same mode of action, which forms the basis

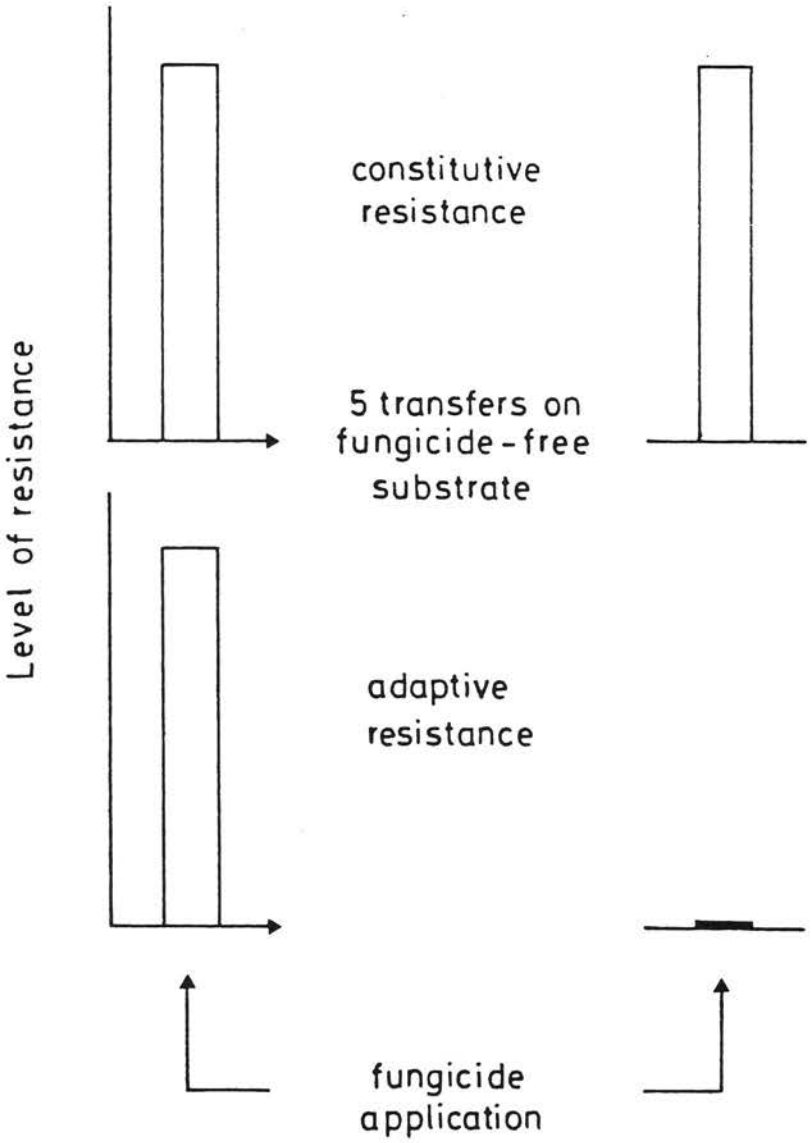

Figure 1. Model of constituve or adaptive fungal resistance after 5 transfers and growth on a fungicide – free substrate.

for cross protection. The phenomenon of cross resistance can usually be demonstrated, but there are exceptions. *Botrytis cinerea* for example reacts to four dicarboximide fungicides quite differently (Table 1). In the agar plate test there is strong inhibition by 10 ug a.i./ ml of iprodione and metomeclan, while procymidione and vinclozoline have no effect on mycelial growth and sporulation at 50 ug/ml. This means, that the first two fungicides could still be used to control *B. cinerea*, whereas the latter two are ineffective.

Factors Determining Resistance Development

A no-effect response after prolonged fungicide application in the field against a hitherto sensitive pathogen results from several complementary factors.

Type of Inhibitor: Fungicides can be divided into multi-site and single-site inhibitors. The first interfere with a number of different reactions in several pathways of the target organisms, while the latter inhibit only one specific step in a biosynthetic process of a pathogen. To completely overcome the toxic effects of a multi-site inhibitory fungicide, a pathogen has to mutate in several genes simultaneously, a rare event in nature. In the case of single-site inhibitors, only one mutation is sufficient to render a pathogen resistant to the action of a fungicide. It is therefore not surprising that all cases of marked fungicide resistance in the field are almost invariably linked to single-site inhibitors, which are represented by all modern fungicides.

Table 1. Growth and sporulation of resistant strains of *Botrytis cinerea* in response to different dicarboximide fungicides (Borge and Schlösser, unpublished)

Fungicide	Concentration in µg a.i. /ml				
	0	5	10	25	50
iprodione	+++	++	+	–	–
metomeclan	+++	++	+	–	–
procymidone	+++	+++	+++	+++	+++
vinchlozolin	+++	+++	+++	+++	+++

Frequency of Treatments: The more treatments per season the higher is the selection pressure of a fungicide and thus the danger of selecting RFS within fungal populations. The threshold appears to be 3 – 4 treatments per season. This is reflected in an investigation on the occurence of RFS of *Gerlachia nivalis* , the causal agent of snow mold of Gramineae, after treatment of golf greens with benzimidazole fungicides (Table 2). With 0 – 1 treatments the percentage of RFS within the fungal population remained rather low, while 4 or more applications resulted in a considerable selection of RFS. This acceleration is not restricted to repeated treatments per season but is likewise of importance in agricultural systems with or approaching monoculture or in horticultural systems, where plants remain on the spot for years. When the same fungicides are applied in such systems year after year, the danger of resistance development will increase accordingly.

Table 2. *Gerlachia nivalis* on golf greens in Hessen and percentage of benomyl-resistant strains in relation to the number of treatments wih benzimidazole fungicides per season (after Huth and Schlosser 1980)

Location	% *Gerlachia*[a] *nivalis*	Treatments per season	% resistant[b] fungal strains
1	67	5	76
2	92	5	76
3	66	12	75
4	45	7	72
5	47	9	68
6	61	10	64
7	86	1	8
8	56	1	3
9	96	0	2

(a) on 100 stems of diseased grasses, respectively.

(b) in the agar plate test with 10 µg a.i. benomyl /ml

Mutation Frequency: Like all other organisms, plant pathogenic fungi have a rate of natural mutations, which are undirected. At a rate of 10^{-6} to 10^{-9} they are inconspicuous within fungal populations in the field. With increased selection pressure, due to application of a certain fungicide, mutations directed against this fungicide have an advantage over sensitive strains and will be selected. When the selection pressure is high enough only RFS, originating from naturally occuring mutants, will survive. In this case we will observe a no-effect response to the treatment.

Fitness: This term denotes the vitality of RFS in comparison to sensitive strains (SFS) and includes several parameter, mainly disease and sporulation efficiency. The first means the rate of fungal development, the second the rate of conidia or spore production. Both should definetely be tested by inoculation of plant material. Measurement of mycelial growth or conidia production on artificial media are of little value in most cases, because the fitness is an outcome of the interactions between host and pathogen. In such investigations one has to differentiate between absolute and relative fitness. For the first, tissue colonization by RFS and SFS and sporulation are studied by inoculation of fungicide-free, seperate plant units. The comparison of the parameters will reveal whether RFS are less fit than SFS, if cultivated independently without selection pressure. For the second, fungicide-free plant tissues are inoculated with a mixture of conidia or spores from RFS and SFS, usually in a 1:1 ratio. After defined periods, the conidia produced are collected and used for inoculation of fresh plant material. This process is repeated several times. The percentage of RFS within the population is determined at each cycle and will provide evidence whether RFS can compete successfully with SFS in mixed populations in the absence of selection pressure by the fungicide. This relative fitness is an important parameter in a critical evaluation and the consequences of a resistance situation.

Strategies to Prevent or Retard Resistance

Our knowledge concerning this aspect is relatively limited. More field experiments are required to test the validity of the proposed concepts.

Prevention: Out of the various possibilities, three points will be mentioned.

One measure could be a reduction in the number of sprays per season or the rate of active ingredient per application. Both are linked to the question whether it is really necessary to control a pathogen close to its eradication. The investigation by Schein et al. (1984) on the control of *Erysiphe graminis* f. sp. *tritici* on wheat with triadimefon provides some data for this point. They tested doses of 0 – 6 mg / 1200 ml water under glass house conditions (Figure. 2) and found that 4-6mg were sufficient to keep the pathogen below the epidemic threshold of about 17% sporulation as compared to the untreated check. Under field conditions the dose will have to be higher, but there is still ample latitude between 6 mg and the corresponding recommanded field dosage of 225 mg. Besides the relatively minor advantage of lesser costs, a reduced application rate could affect the level of resistance significantly. Provided RFS have a lower fitness, the SFS will suppress the development of RFS within the fungal populations. Under such circumstances a use of the fungicide could be extended for quite some time. This concept is convincing, but has still to be validated by corresponding field experiments. Similar investigations with other host-parasite-systems are indicated. Further alternatives to suppress resistance development could be fungicide alteration or combination of several active ingredients. In both cases the products should have a different mode of action. Alteration would always be preferable over repeated sprays of the same fungicide or products with the same mode of action, respectively, but it appears that combinations are most promising. From the few available data the model of *Cercospora beticola* management will be presented as an example. Delp (1) compared the development of RFS of this leaf spot fungus on sugar beets after application of benomyl singly and in combination with maneb. The combination was either used from the onset of the investigation or 1,2 and 3 years, respectively, after treatments with benomyl alone (Figure 3). When only

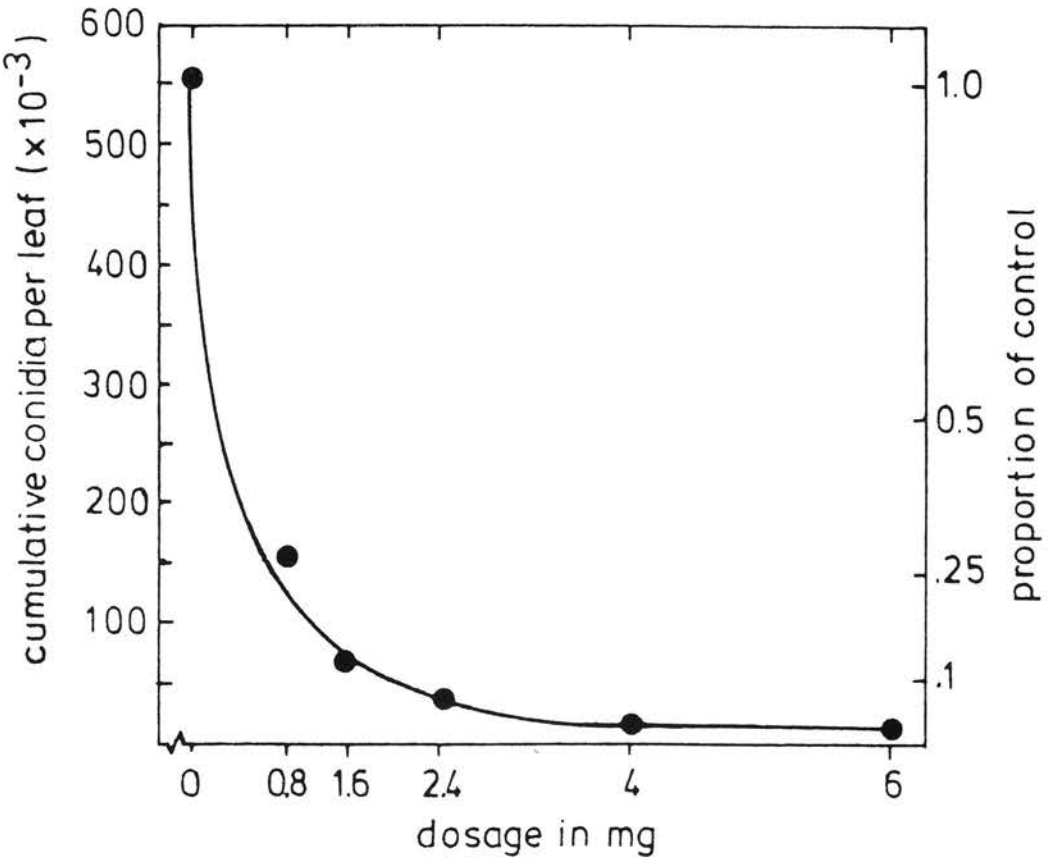

Figure 2. Total conidia per leaf produced by day 13 after inoculation with *Erysiphe graminis* f. sp. ***tritici*** as affected by six dosages (milligrams per 1200ml) of triadimefon (after Schein et al. 1984).

benomyl was applied, a high frequency of RFS appeared within three years. When the combination benomyl-maneb was used right from the beginning, selection of RFS was retarded almost indefinitely. A use of the combination 1.2 or 3 years later still retarded the selection of RFS, but to an increasingly lesser extent. This means, that newly developed fungicides should be used in combination with appropriate fungicidal counter-parts from the time when they are first introduced to the market. A single-site inhibitor, such as benomyl, should preferably be combined with a multi-site inhibitor, such as maneb.

Overcoming: What can be done in cases where high percentages of RFS within the populations result in a no-effect response to a fungicide? The easiest way out would be the switch to another fungicide with a different mode of action, provided there is such a product. It would be wise to combine this alternative with an appropriate fungicidal counterpart as a safeguard against future resistance development. In Central Europe, where *Erysiphe graminis* on wheat and barley has become resistant to triadimefon or related triazols, fenpropimorph is used instead, again without a safeguard. When there is no alternative fungicide available, resensibilization could be the last hope. This term implies, that the level of RFS will steadily decline after discontinuance of a resistance promoting fungicide for some years. Provided the RFS have a lower fitness than SFS, the rate of the first will decline to such an extent, that the discontinued fungicide could be used again. This is an interesting concept, but will seldom be applicable. It has been studied in regard to benomyl-resistant strains of *Botrytis cinerea* on grapevines. After 5 years of discontinuance, the level of RFS was still at about 80% as on the onset of the study. The RFS were apparently of equal fitness with the SFS, excluding resensibilization as an effective measure. This phenomenon might, however, work in the case of resistance of the same fungus to dicarboximide fungicides. These RFS appear to be less fit, which opens the chance to test the concept for its validity. Field studies are under way, but final conclusions cannot yet be drawn.

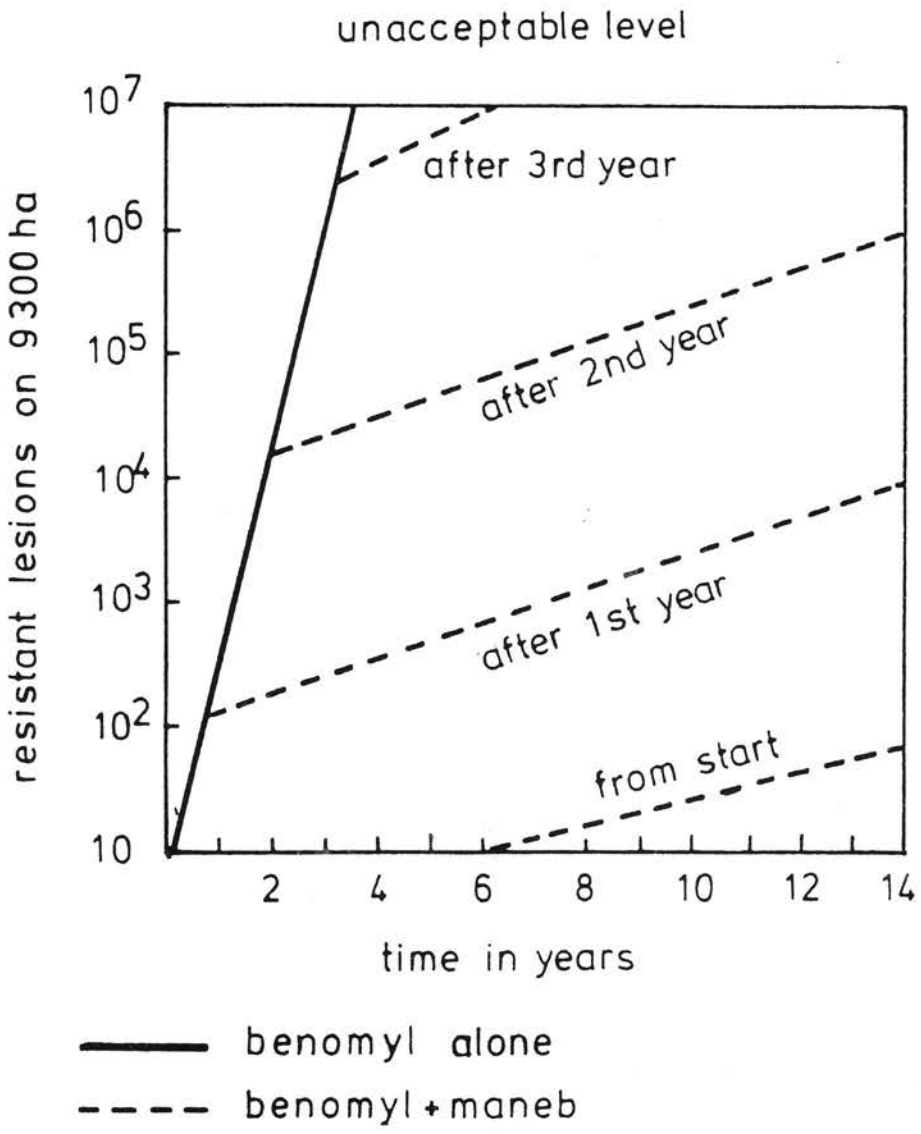

Figure 3. Development of resistant fungal strains of *Cercospora beticola* in response to treatments with benomyl alone or combined with maneb (after Delp 1980).

الملخص

شلوسر، ايكارت. 1986. مشكلة المقاومة ضد المبيدات الفطرية لدى الفطريات الممرضة للنبات. مجلة وقاية النبات العربية 4 : 120 - 124

إن تطور المقاومة للمبيدات الفطرية لدى الفطريات الممرضة للنبات ظاهرة عالمية الانتشار. ستعرض العوامل المسؤولة عن تطور المقاومة، اعتماداً على أمثلة مختارة. كما سنناقش وسائل التغلب على مشاكل المقاومة الموجودة فعلاً وكذلك الخطط للوقاية من أو تأخير تكاثر مجتمعات فطرية مقاومة للمبيدات الفطرية.

كلمات مفتاحية: فطريات، مقاومة المبيدات.

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