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CONTROL OF BOTRYTIS DISEASES

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Introduction

This session is to be devoted to a particular group of fungi, Botrytis. This makes it of particular interest to those of us from Scotland where we have something like 7,000 acres of raspberries concentrated in the Dundee, Perth Blairgowrie, Arbroath area. In addition we have a substantial strawberry growing industry. As you know, we have already made substantial progress in our work on the Virus diseases of these two crops, but practically no progress at all has been made towards reducing the very serious losses that can occur from Botrytis in epidemic years. There is always some loss of crop due to Botrytis. It may be 5 - 6 cwt of strawberries per acre and 6 - 7 cwt of raspberries but in epidemic years, which occur at fairly short intervals, the loss is one to one and a half tons per acre. The potential market for a suitable fungicide should be seen against the background of yields and values. An average crop of raspberries produced by a good grower is from $2\frac{1}{2}$ to 3 tons per acre and with raspberries £95-100 per ton this gives gross income of £300 per acre and a loss of some income in epidemic years of £100 per acre. While it is true that some of the standard fungicides will give some control they cannot be used because they make the fruit unacceptable to the public.

It is against this background that we wish you to examine the problem of Botrytis, the diseases it causes and the present state of means for its control under field conditions.

Discussion

Q. Mr. R. W. Marsh

How does the development of resistance to the dithiocarbamates differ from that to the chlorinated nitrobenzenes?

A. Dr. R. K. S. Wood

There are a number of important differences. In the first place, a large proportion of Botrytis cultures exposed to the vapours of the chlorinated nitrobenzenes or to dicloran develop resistance relatively quickly, within 10-14 days. Secondly, the resistance which develops is, in some respects, almost complete because resistant strains grow almost as quickly in the presence as in the absence of the fungicides. However, most resistant strains do not sporulate in the presence of the fungicides although they do so freely once the fungicide has been removed. But we have obtained some resistant strains which will sporulate in the presence of the fungicides.

Another difference is in the way in which the resistant strains are produced. Those resistant to dithiocarbamates were produced only after a relatively long period of training in which the fungi were exposed to increasing, but sub-lethal doses of the fungicides, whereas the other strains appeared "spontaneously" after growing for some time in the vapour of the fungicides.

Undoubtedly, however, the most striking difference is in the order of resistance to the fungicides. In fact, I know of no other group of fungicides used on plants where resistant strains are so little affected by the fungicides.

Q. Dr. J. T. Martin

Recently reports have been made of the production in plants of antifungal agents in response to infection. Does Dr. Wood think that this occurs following Botrytis attack?

A. Dr. R. K. S. Wood

I think there is some evidence that this happens. We have done some work in this connection with the disease of broad beans caused by Botrytis fabae, and obtained some evidence for the development in bean tissues of substances formed following the oxidation of phenols which reduce the activity of the pectic enzymes secreted by B. fabae. And, of course, there is the evidence from similar work which has been done at Long Ashton. Such substances are really formed as a response to infection; I believe that it is fair to say this because the fungus kills cells of the host thus permitting phenols in the cells to be acted upon by the polyphenolases of the host or the fungus - the substances then formed inactivate the pectic enzymes of the fungus.

Q. Dr. E. Evans

Could you please comment on what you consider to be the main factors which determine whether a given infection by Botrutis becomes aggressive? Is inoculum potential one of these factors?

A. Dr. R. K S. Wood

I believe it is important. For example if you take healthy lettuce leaves and put drops of water containing spores of B. cinerea on the surface there is a fair chance that only a limited, leaf-spot type of lesion will be produced. But if the spores are suspended in a dilute solution of a readily available carbohydrate such as glucose, a rapidly spreading, aggressive type of lesion will be caused. You can also increase the chances of getting an aggressive type of lesion by increasing the number of spores in the drop, and by increasing the water content of the leaves. But certainly the most striking effects are produced by readily available carbohydrates. Incidentally, even with the same types of treatment one can get aggressive and non-aggressive lesions on the same leaf.

Q. Dr. J. B. Loughnane

Can Dr. Wood say if the species of Botrytis which attacks onion foliage is the same as that which causes rot of the bulbs? In my experience onion foliage is frequently attacked by Botrytis especially when the crop is grown in soils deficient in potash.

A. Dr. R. K. S. Wood

My statement that B. allii does not grow profusely on plants in the field was based on remarks to this effect in the literature, and on my own observations on small plots at our field station. But I am entirely open to correction on this point. However, even if B. allii is common on growing plants, it still puzzles me where the fungus comes from in the first place, particularly when the crop is grown on land which has not carried onion crops for some time.

Q. Mr. D. Rudd-Jones

Dr. Jarvis has suggested that a systemic fungicide might be expected to provide the most effective control of Botrytis on strawberries and raspberries. Surely, with a disease that kills the host tissue quite rapidly, it is unlikely that a systemic compound will control the disease unless it is applied and has become systemically distributed in sufficiently high concentrations to provide control before infection occurs?

A. Dr. W. R. Jarvis

Yes, I think a systemic fungicide would only be of value if it is applied before infection and in this case that would mean applying it at the pre-blossom, closed bud stage. We have in fact tried one systemic fungicide which has been shown to be reasonably good in glasshouse crops but we have had very patchy success in field soft fruit crops. It was first applied at the time when the fungicides are normally applied i.e. at the petal-fall stage, and it would appear that this was too late to be effective, even assuming that a sufficient concentration was present.

Q.

Recent work in Oregon has shown that infection of strawberry first occurs at a very early stage in its development and that it then remains symptomless, often until it is ripe, the fungus mycelium remaining in a quiescent stage. Has Dr. Jarvis confirmed this, and is there a similar behaviour in raspberries?

A. Dr. W. R. Jarvis

Yes, I have confirmed Powelson's observations in Scotland and shown it to be true to a much greater extent in raspberries. We are still working on the mechanism which holds the fungus in check. It does not appear to be the same that Dr. Wood has recently described in bean leaves, but is possibly bound up with some substance which is present in green fruit but not present in the ripe fruit. It operates in both strawberries and raspberries.

Q.

Could Dr. Jarvis say whether green fruit is always completely symptomless, and if so, is the tannin in green fruit acting as a fungistatic agent?

A. Dr. W.R.Jarvis

It is not quite true that green fruit is always symptomless; one can in fact rot green strawberries, but it is not easy. The rot occurs occasionally under field conditions but usually escapes notice because it is generally concealed beneath the calyx. It appears only under warm conditions with temperatures well over 20° C. Regarding the tannin content of the green fruit, it is quite true that strawberries do contain an appreciable amount of tannin but so far as I know there is as much tannin in ripe fruit as in green fruit. We may have a case such as Dr. Wood describes for potatoes. If you kill the tissue, the fungus will grow on this and on an extract made from green fruit quite readily. It appears possible that some physical mechanism operates in the fruit, or a chemical mechanism, or possibly a combination of both.

Q. Mr. R.G. Pawsey

Botrytis damage is sometimes important in forest nurseries, particularly on spruce. Almost invariably the damage by the fungus follows physical damage, usually by frost. The frequency of frost in forest nurseries makes it extremely difficult to control Botrytis damage by spraying with contact fungicides and we are very interested in the development of suitable systemic materials. Could Dr. Jarvis give more information on the systemic fungicide he mentioned?

A. Dr. W.R.Jarvis

The material is the antibiotic, griseofulvin, and this is the only one of its type used. The results were variable possibly because the time of application was wrong; the infection may have occurred before the fungicide was applied. This work was done before we really appreciated that this very early infection occurred almost as soon as the buds were open.

Q. Mr. R.G. Pawsey

Griseofulvin has been used in forest nursery experiments with poor results. Can anybody give information on the use of cycloheximide against Botrytis infection?

A. Dr. W.R.Jarvis

I have no personal experience of the material. It has been used in the States but only as a post-harvest treatment in order to preserve soft fruit in storage and transit. I do not know of any work in which it has been used in the field.

THE BIOLOGY AND CONTROL OF DISEASES CAUSED BY BOTRYTIS SPP.

by R. K. S. Wood
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It is difficult to assess the losses caused by Botrytis spp. because of the variety of the crops which are attacked, the nature of the diseases which are caused, and because the incidence and severity of these diseases depend so much upon the weather and the way in which the crops are grown. There can, however, be little doubt that in many years, Botrytis cinerea, or one of the allied species, is the cause of major loss of lettuce, broad and field beans, onions, raspberries, strawberries, and various bulbs and corms. It can also be very troublesome on many other crops, particularly glass-house ornamentals, and although few systematic studies have been made, B. cinerea is probably one of the main reasons why in many seasons we find it so difficult to harvest good quality seed in this country. The total losses in the United Kingdom each year must still amount to many hundreds of thousands of pounds in spite of the attempts which are made to reduce them by the use of fungicides.

The diseases may be conveniently divided into two groups. In the first are those in which the fungi responsible are relatively specialised in their parasitism and are sufficiently distinctive, particularly in their conidiophores and conidia, to be recognised as species distinct from B. cinerea. Thus we have B. allii and B. byssoidea mainly parasitic on Allium spp., B. fabae mainly on Vicia faba but also on other legumes, and B. polyblastis largely confined to Narcissus. A number of these species have a perfect, apothecial stage which is not uncommon under natural conditions. In the second group are the many diseases caused by the aggregate species B. cinerea which probably has a host range as wide as any other fungus we have in this country. The species has a large number of forms which differ considerably in a variety of ways. Further study of some of the more distinct of these may justify their elevation to specific rank. Forms recognised as B. cinerea have been shown to have Sclerotinia fuckeliana as the perfect stage but apothecia seem to be rare in the field. Most forms and species produce conidia abundantly on their natural substrates, though they often do so far less readily in culture. Sclerotia are common in the field and are almost always produced abundantly in culture. A proportion of the conidia remain viable under ordinary laboratory conditions for many months, and the sclerotia are also long lived. Microconidia, much smaller than the conidia, may also be produced freely and function sexually before apothecia are formed.

The various diseases have a number of features in common. Although most species can probably invade and become established in healthy parts of plants, they do so more readily when the tissue being invaded is senescent, moribund or dead. Most of the primary infections during the growing season are made by the germ-tubes of conidia. The progress of the parasite after invasion depends on the type of tissue, its condition, and the surrounding temperature and humidity. In massive invasions of

relatively large bulks of storage tissue the rate of advance of the fungus is largely determined by temperature and by the nature of the tissue. At suitable temperatures, some tissues, e.g. those of apples, are rotted quite quickly. In others the spread of the fungus is relatively slow and not nearly so rapid as might be expected from the growth rate of the fungus on killed tissue in vitro. Thus, although B. allii moves continuously through onions so that ultimately the bulb is destroyed, it nevertheless moves slowly and cannot be regarded as an aggressive parasite under normal conditions of storage. The rot in onions, as in other tissues in which the fungus grows relatively slowly, has not the typical characters of a soft rot. We are at present studying this as well as other aspects of neck rot of onions.

When Botrytis spp. attack leaves their progress again depends on the nature of the tissue and on temperature, but now it is often much influenced by the water content of the tissues and the relative humidity of the air around the plant. A high relative humidity and, probably, free water is necessary for invasion and establishment and unless humid conditions persist the lesion very often fails to develop and the fungus does not sporulate. This is well seen in the typical spot lesions found on broad bean leaves infected by B. fabae, and on tulip leaves infected with B. tulipae. Both resemble those caused by more typical leaf spot pathogens such as Ascochyta spp. and Septoria spp. The spot character of the lesions is lost under certain conditions, particularly when the leaves are kept in a very high relative humidity so that their moisture content is probably higher than usual. The pathogen then spreads rapidly to cause a soft rot upon which it sporulates freely at a later stage. At present we know very little about the mechanisms that regulate the two types of lesions but the problem is of considerable interest both academically and practically because generally it is the spreading, aggressive type which is the more damaging in the field.

Botrytis spp. often attack stems. When they are soft and succulent, as in broad bean, the course of infection resembles that in leaves, but in more woody stems the attack generally takes the form of a die-back from the top of the shoot or occurs as a lesion lower down. The fungus moves relatively slowly through the tissues, although it generally sporulates very freely on the parts that have been killed. The slow movement through the woody parts of the stems is readily understood because of the nature of the substrate which has to be destroyed, but it is rather puzzling why the fungus does not move more readily through the softer tissues outside the cambium.

Now that I have given a brief outline of the ways in which Botrytis spp. attack higher plants, I should like to say a few words about resistance to infection and colonization by these fungi. Although conditions can be found in which almost every type of plant will succumb to attack by one species of Botrytis or another, it is often surprisingly difficult to get aggressive lesions established on well grown plants in "normal" conditions of humidity and temperature. Although the fungus may become established in such

plants, the lesions remain very small. Very little indeed is known about this very common and important type of resistance which is readily broken by comparatively minor changes in the external environment of the host. It is unlikely to be structural in character, or to depend upon substances already present in the tissues and which directly retard the growth of the parasite in vivo. I think it more likely that it depends on substances which are formed in the plant as a result of infection, and which interfere not so much with the internal metabolism of the pathogen as with the various toxins and enzymes by which the pathogen damages and kills the host tissue. The progress of the lesion will be determined by the rate at which such substances are produced and how long they retain their activity. Resistance will depend upon how quickly inhibitors of these substances are formed in the diseased tissue. In a balanced system of this sort, it is easy to see that slight alterations in the condition of the host might have cumulative effects which would profoundly alter the progress of the lesion.

Coming now to disease control, somewhat different problems are presented by the diseases caused by the more specialised species and by B. cinerea, although the differences are of degree rather than kind. When the parasite has a restricted host range it is at least feasible that the disease in a particular area could be greatly reduced in severity or eliminated by growing crops in clean soil and by planting only healthy material, or material which has been treated by heat or with fungicides so as to make the parasites innocuous. On a small scale disinfection of contaminated soil with fungicides might be practical. With these diseases there is the advantage that at the beginning of the season the sources of the inocula can be known with some certainty and are not likely to be numerous provided reasonable precautions are taken. "Fire" of tulips, caused by Botrytis tulipae may be placed in this group because for all practical purposes it is confined to the genus Tulipa.

At the other end of the scale there are the diseases caused by B. cinerea. Many forms of this species which are important pathogens will have a wide host range among cultivated and wild species present in areas where crop plants are likely to be grown. This make the problem of control much more difficult because no matter how much care is taken to reduce the amount of fungus associated with the crop plant, there will always remain a reserve of inocula in the environment around the crop which might attack the crop when conditions become suitable. And, of course, not much that is practical can be done to reduce their number. So even if the crop is planted on clean soil and the planting material is free from the fungus, one must assume that the crop will be attacked when temperature and humidity are suitable.

While it is easy to see why B. cinerea should be so prevalent on susceptible crops whenever conditions are suitable, the fact that the same can be said of diseases such as neck rot of onion caused by B. allii is less easily understood. This species is more or less confined to Allium spp. and most onion crops are grown from seed, so that unless the crop is grown on contaminated soil there should be very little or no introduction of the fungus with the crop. Moreover, the fungus is not particularly

evident on the growing crop even in wet seasons. It is, therefore, somewhat puzzling, at least to me, that neck rot is so often a major source of loss. I should be grateful for enlightenment on this problem.

Before dealing with the inactivation of Botrytis spp. with the more conventional types of fungicides, I should like to consider very briefly some other ways of controlling disease. There is, of course, the obvious one of not using contaminated or infected planting material. And because the fungi grow and sporulate freely on dead and moribund tissue, it might always be possible and with little extra effort to grow plants so that less of this type of tissue is produced. Colonization of any that is produced might be reduced by growing plants so that the moisture content of this tissue is kept at levels which preclude attack, or by encouraging a growth on it of saprophytes which will exclude the pathogen. There is a variety of ways in which this can be done in different crops, and there are examples in the literature where the control which has been obtained by cultural methods can be explained by one or other of these mechanisms. The potential advantages of these cultural and biological methods of control are, of course, that they need not add appreciably to the costs of production (they will not be adopted if they do) and they avoid the trouble of unpleasant and toxic residues in crops such as lettuce and strawberry. A disadvantage is that these methods are likely to be unreliable unless they are developed on a rational basis so that the mechanisms involved are pretty well understood. This will call for a much greater knowledge of the biology of Botrytis diseases than we now possess, and it will be a long time before this is available even if determined and systematic efforts are made to obtain it. Also, it is unlikely that biological and cultural methods will be fully effective even under favourable conditions. Because of this they would be best developed as adjuncts to the more conventional methods which use fungicides. This brings me conveniently, and with not too much time left, to my last topic, and the one which will be of greatest interest to most members of this audience - control with fungicides. It was my deliberate intention not to deal with this in any detail because I know that this is to be done by later speakers. There are, however, a few aspects I should like to mention briefly.

It is somewhat surprising that some fungicides, active against Botrytis spp. in vitro and which control foliage diseases caused by other pathogens, are relatively ineffective against Botrytis spp. in the field. I have in mind copper fungicides and in particular Bordeaux mixture, which do not seem to have been very successful in the control of Botrytis diseases. It might be, of course, that these fungicides are effective to start with but their phytotoxicity creates a new lot of damaged tissue on which residual fungus can multiply. The net result may be no control, or even an increase in the amount of disease. But this is unlikely to be the whole explanation. Another factor might be the inability of some fungicides to penetrate tissue which carries the pathogen internally. If such tissue were abundant, a fungicide which acts on the surface would only reduce the quantity of inocula temporarily. The inactivation, possibly by adsorption, of some fungicides by dead plant material containing the pathogen is also a possib-

ility and no doubt others will suggest themselves to you. Having said that I cannot understand why some fungicides do not control these diseases. I should now like to present the reverse side of the picture because I am also puzzled why certain others do control disease. I have in mind the chlorinated nitrobenzenes and related compounds which have been reasonably successful against Botrytis diseases. Until fairly recently this was their main use. Pentachloro-nitrobenzene, for long one of the most widely used members of the group, has not much effect on spore germination except to delay it slightly, and the fungus continues to grow on agar in the presence of high concentrations of vapour of the fungicide although at a reduced rate; 2,6-dichloro-4-nitroaniline is not much more effective. It is only the third of the extensively used of these compounds, 2,3,5,6-tetrachloronitro-benzene, which has any pronounced effect on spore germination and growth. Even this substance does not prevent spore germination. The one thing which can be said in favour of the three substances so far as in vitro tests are concerned is that each retards or prevents sporulation so long as the fungus is kept in sufficiently high concentrations. This effect is quickly lost when the fungicide is removed. From all this one might anticipate some control of disease in confined spaces in which quite high concentrations of vapour could be maintained, but in practice this cannot happen very often except perhaps in frames. In glasshouses and in the field the concentrations required for activity in vitro would only rarely be obtained, and it is difficult to see how these fungicides could be very effective as protectants, eradicants or anti-sporulants under these conditions. It is possible that they are effective not because they act directly upon the fungus but because they alter the host plant so that it becomes resistant. This would not be altogether surprising because in sufficiently high concentrations they can greatly affect the growth of plants. For example, penta -, and tetrachloronitro-benzene can retard the growth of lettuce without causing any other striking damage, and, of course, their ability to suppress sprouting of potato tubers in storage is well known and has some practical advantages. I have also wondered whether they are the sort of substances which would accumulate in the cuticle to concentrations which would be sufficient to reduce significantly the rate of growth of penetrating hyphae.

There remains another reason which makes the effectiveness of these fungicides rather surprising. In culture Botrytis spp. readily and fairly quickly produce strains which are almost completely resistant so far as vegetative growth is concerned. We know that resistant strains of B. allii retain their pathogenicity to onions and we are about to study the pathogenicity of other strains. All resistant strains retain their ability to sporulate, but most produce few or no spores in the presence of high concentrations of the fungicides. There are, however, some strains that do sporulate under these conditions. So far there is little evidence that resistant strains are of any practical importance. The place to look for these would be on crops where the use of chlorinated nitrobenzenes has not given so good a control as was expected. Mr. D. C. Gwynne and Mr. D. R. Humphreys Jones very kindly sent me lettuce and tomato plants from crops where this had happened but I found no evidence of resistance in

isolates of B. cinerea from this material. So we still wait for good evidence of resistance in the field to these as to other fungicides and in this respect at least we are more fortunate than our entomological colleagues.

This completes the detail of what I have time to say but it would be inappropriate in a conference of this sort to end on too optimistic a note. Speaking as I do from the sidelines, because I am not involved directly in the business of controlling diseases, it seems to me that at present there remain a number of diseases caused by Botrytis spp. which are not so readily or efficiently controlled with fungicides as are certain other diseases. It may be that we need better fungicides or ones which act differently from those now in use. But I also think it possible that we may ask too much of any fungicide to control some Botrytis diseases when conditions are particularly suitable for their development. The task set for the fungicide might be made considerably easier by the judicious use of cultural methods aimed at reducing the amount of dead tissue which is, or can be, colonized by the fungi. The function of the fungicide would then be to complete the job of control.

PROBLEMS IN THE CONTROL OF RASPBERRY AND STRAWBERRY GREY MOULD

by W. R. Jarvis
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During the last decade many fungicides have been used in attempts to control grey mould of raspberries and strawberries caused by the fungus Botrytis cinerea. In no case has even a moderately successful material been found which is not the subject of one or more serious objections when applied at the time of flowering and fruiting. Many fungicides, particularly when formulated as dusts, spoil the appearance of the fruit, one or two may be phytotoxic (e.g. dichlone and thiram (Cox and Winfree, 1957)), some may cause tainting, particularly in processed products (e.g. captan and thiram, (Marsh, Martin and Crang, 1955 and Crang and Clarke, 1961)), and all are subject to regulations regarding tolerated levels of residues.

A notable feature of the various field trials of prophylactic fungicides has been a marked lack of consistency in results. Only infrequently has a good level of disease control been attained, and results generally vary quite widely from year to year, even on the same plots, using the same fungicide and as nearly as possible the same rates and times of application with respect to crop development. More usually, the disease incidence is reduced by the best of the fungicides to only a third or even a half of that on the untreated control plots.

An examination of the pattern of crop development, of the biology of the parasite and of the techniques of fungicide application suggests some reasons for these variable results.

Both strawberry and raspberry fruits mature over a long period and, at any given time, flowers and fruit in all stages of development are present together. The normally recommended time for the first fungicide application is at petal fall, a rather poorly-defined term in view of the long period over which this occurs. It is, however, possible to discern a period of maximum petal fall, but at this stage some fruit is already ripening, while some buds are still very young and tightly closed. The recommended practice of two or three applications at 10-day intervals, inevitably contaminates considerable quantities of ripening fruit, but fails to protect the fruit developing from still closed buds.

Assuming that it is possible to tolerate a fungicide on maturing fruit, there are also almost insuperable physical and economic difficulties in maintaining a complete prophylactic cover on rapidly expanding fruit surfaces and Jarvis (1961) found that, in general, no better control was obtained by continuing fungicide applications throughout the picking season, than by stopping treatments about ten days before the first pick.

The problem in grey mould control are emphasised when the pattern of fruit infection is considered in relation to the biology of the parasite. While B. cinerea generally sporulates on organic debris in fruit plantations throughout the year and especially during the flowering and fruiting period, comparatively few infections can be ascribed to the text-book

mode of infection, i.e. by the germination of spores in water droplets on the surface of the fruit (Blackman and Welsford, 1916). Despite airborne spore concentrations of the order of 10^4/cu.m. frequently during the fruiting period (Jarvis, 1958), only about 1% of all fruit infections have been found to result directly from spore germination (Jarvis, 1961). Unfortunately it is this type of infection which is probably the most easily controlled by fungicides applied to the fruit surface. In a strawberry plantation (var. Talisman) in 1960, 64% of infections were found to result from the penetration by hyphae growing from adhering infested organic fragments, such as moribund petals, pieces of straw and weed debris, 18% from contact with rotting fruit, and 17% had been initiated from the calyx end. Because of the relatively cleaner conditions in raspberry plantations, there was virtually no infection of fruit from adhering organic debris. Petals are shed as in strawberries, but rarely adhere to the fruit for long enough to permit infection; the microclimate of the fruit and its surface contours do not favour petal adherence. Infection by contact with rotting raspberries and especially with rotting receptacles remaining after the berries were picked comprised 35% of infections, while 64% were initiated from the calyx end.

In strawberry plantations the problem of infection from infested organic debris is particularly important. In the variety Talisman, more than a quarter of a pound of such material consisting of dead leaves, petioled, stolons and straw, has been removed from a single crown in a plot which had been completely defoliated after the previous harvest and the debris raked off. Considerably more debris remains in non-defoliated crowns. Attempts to control the fungus in its over-wintering stage by applying a formulation based on phenyl mercury oxine, as a drench, in January and February have not yet been successful, and it is assumed that reinfestation of the debris occurred before the fruiting period. Colonisation of debris at any time of the year is rapid, and even straw newly laid as a mulch may become infested within 14-21 days. It seems most unlikely that any fungicide good enough to eradicate the fungus shortly before picking would be tolerable from the toxic hazard viewpoint. A small reduction in infection was obtained, however, in plots of the strawberry Talisman in which the straw mulch was previously treated with a waterproofing and fungicidal preparation of copper 8-quinolinate and also when black plastic sheeting or wet-strength paper impregnated with copper 8-quinolinate was substituted for straw. Some improvement in plantation hygiene by removing debris could be effected where post-harvest burning is practicable, but because of the climate, this has not yet been possible at Mylnefield.

The infection figures also emphasise incidentally the need to keep fruit picked frequently and, if possible, to remove infected berries.

Infection from the calyx end of the fruit is very important in both crops, and may result from one or both of two processes. Firstly, spores may be trapped between the sepals and the fruit surface in a film of water and germinate directly to infect the fruit, and secondly, infection of attached

moribund flower parts may occur, followed by infection of the fruit from an inoculum having a large saprophytic food base and therefore a greater inoculum potential, sensu Garrett (1956). A high inoculum potential also accounts for the striking success of infections from infested debris adhering to the fruit surface (Brooks, 1908). Even where spore germ tubes penetrate directly, their effect may be enhanced by the presence of solutes from moribund material (Brown, 1922). The relative importance of the two modes of calyx-end infection is unknown, and perhaps rather difficult to assess.

At all events, the airborne spore inoculum is of particular importance in initiating infestations at the calyx end and small scale experiments at Mylnefield with an antisporulant, hexachloro-2-propanol (Horsfall and Rich, 1960), alone and in combination with captan, to reduce the incidence of airborne spores have not been encouraging. In view of the considerable distance travelled by spores of B. cinerea it is unlikely that this approach will be effective, except possibly in isolated plantations.

Infection of strawberry flower parts occurs at a very early stage in development and they remain largely symptomless until the fruit begins to ripen. In 1961 isolations made almost a month before picking from scarcely-opened flower buds showed that 3.1% of carpels were already infested, 1.2% of the stamens and 2.0% of the sepals. These figures were approximately doubled in slightly frosted flowers. At the same time, 57.8% of detached petals adhering to other developing flowers and fruit were infested. A month later the infestation rates in newly fertilised flowers were: carpels 20.8%, stamens 17.0%, sepals 3.4% and undetached petals 3.1%. Corresponding figures for slightly-frosted flowers showing no grey mould symptoms were: carpels 26.0%, stamens 48.1% and sepals 5.3%.

For raspberry flowers and fruit the figures were even higher; 39.1% of all floral parts of unfrosted flowers and 49.5% of slightly frosted flowers, were found to be infested.

It has been recently reported from the U.S.A. (Powelson, 1960) that a latent mycelium is often present in apparently healthy green and ripening strawberries, particularly at the calyx end. This mycelium may remain quiescent in the fruit, even until after marketing, but is always potentially aggressive from the white fruit stage onwards. This finding has now been confirmed at Mylnefield and a latent mycelium has also been demonstrated in raspberries. The conditions inducing the change from quiescent to aggressive mycelium are still largely unknown; the physical and chemical changes in the ripening fruit are undoubtedly important, but there is reason to believe that these are not the only factors concerned. It seems probable, however, that the latent mycelium originates from infection of the floral parts at a very early stage in fruit development and the problem of controlling calyx-end infection appears therefore to be largely one of controlling flower infection from the closed bud stage onwards.

It is evident that the normally recommended time of the first fungicide application is probably too late for prophylaxis, and that the internal

flower parts should be protected as soon as they are exposed. The difficulties of achieving this when flowers do not develop simultaneously have already been mentioned and there are also technical difficulties in adequately protecting partially covered flower parts in opening buds.

The fungicides commonly used for the control of grey mould have been shown to be quite effective against the fungus in other crops, e.g. in glasshouse lettuce, but are frequently disappointing in strawberry and raspberry crops. The application of fungicides to mature fruit plantations often necessitates light equipment and low volumes, and it seems likely that these methods do not ensure adequate coverage of all the flower parts. Recent work with strawberries in Germany (Müller, 1961a, 1961b) suggests that very high volumes and high fungicide rates combined with an improved technique, might bring about considerable improvement in control. Thus thiram applied at the rate of 4.8 kg/ha (4.4 lb/acre) (the normal rate being 2.0 kg/ha) in 2,400 l/ha (218 gall/acre) reduced infection from 52.1% to 4.8%. The spray was directed well into the plants from the top and both sides by a triple arrangement of jets, the emphasis being on obtaining a thorough drench. Similar recommendations for strawberry grey mould control in the U.K. have been made by Moore (1961). Captan and dichloronitroaniline applied at Mylnefield in 1961 at rates comparable to those used by Müller, but without the special arrangement of jets, did not give significantly better control than the normal low volumes in strawberries, but in raspberries where a captan drench was directed straight into the young flowers and fruit, infection was reduced from 14.2% to 8.6%. This reduction, however, by Muller's standard is still poor.

It might be supposed that systemic fungicides would give some protection to the flowers and fruit without the necessity of drenching, but many attempts to control the disease with applications of griseofulvin to the flowers and foliage have not been encouraging in either crop.

It is evident that the overall problem of grey mould control in soft fruit may be subdivided into a number of problems, each of which requires a separate approach in considering both fungicide and technique.

General plantation hygiene is of great importance in reducing the potential inoculum in the form of overwintering mycelium and sclerotia in organic debris, which also supports the actively saprophytic and sporulating stages of the fungus. In plantation hygiene may be included weed control, so that at once both an important incoulum source and, particularly in the case of strawberries, an environment conducive to increased sporulation and infection is removed. Attention to conventional methods of plantation management thus can do much to increase the efficiency of fungicides. It seems most likely that when presently available fungicides are carefully selected with respect to the biology of the crop and of the pathogen and of the two in combination, and are applied by improved techniques, at the biologically correct times, control of the disease could be achieved economically and without detriment to the quality of the crops.

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OBSERVATIONS ON THE CONTROL OF BOTRYTIS IN ANEMONES

by A. Elizabeth Jeff
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Summary

Botrytis cinerea will attack Anemones at times during the season when the plant has suffered damage during adverse weather conditions in autumn and winter, or when the plants have been previously weakened by Peronospora infection during summer and autumn and when the weather favours the spread of the fungus.

In trials over a period of four years spraying with zineb, captan and thiram every fourteen days from September to March showed good but variable results. In two seasons captan gave the best results in keeping plots free of Botrytis during the winter months following bad weather, but in the other two years zineb proved the better fungicide. In this case a high incidence of Peronospora occurred in summer and autumn and zineb had given more protection from this disease increasing the plants resistance to frosts with a consequent lower incidence of Botrytis infection.

Tecnazene dust, streptomycin compounds were found of little value, and all compounds containing copper caused severe plant damage.

Dicloran appears promising, the spray formulation being better than the dust.

Introduction

The season of growth of the Anemone plant in the South West is from June when the corms are planted, through the autumn and winter given good weather conditions until March or April. Flowers are produced more or less continuously from September onwards. Thus the Anemone, growing as it does through the winter months is often subject to severe gales and the occasional frost interspersed with periods of mild humid weather. It is at these times when attack by Botrytis can be widespread and serious. Another factor which must be taken into consideration is the amount of Peronospora ficariae (Anemone Downy Mildew) present. This disease spreads in humid conditions in summer and autumn, considerably reducing the plant's resistance to bad weather and consequent Botrytis attack. Being a systemic fungus which slowly reduces the vigour of a well grown plant after secondary infection it is not always possible to tell in the field whether P.ficariae is present or not. Neither the summer or winter sporing stages can be positively identified without the use of a hand lens and in many cases these spores will not be present although the plant is infected.

Treatments

Fungicides which have been in constant use since the trials commenced in 1956 are:-

Zineb at 2 lb of a 65% material in 100 galls water/acre.
Captan at 2 lb of a 50% material in 100 galls water/acre.
Thiram at 2 lb in 100 galls water/acre.

In each season spraying was commenced at the end of August and continued at fourteen day intervals throughout the season weather permitting.

Results

The results of the trials are assessed on the number and quality of flowers and the time at which they are cropped. Visual observations on the general appearance of the plants were made, but correct estimations of the amounts of Botrytis and Peronospora present could not be made without microscopic examination of individual plants facilities for which were not available.

The tables below are the results of the trials of four seasons set out to show the numbers of good quality flowers cropped in successive four week periods from plots of 160 corms. The total numbers in general vary somewhat from year to year but this is only due to differences in actual growth rates in different seasons.

Numbers of good quality blooms cropped in successive 4 week periods. Plots of 160 corms

1956 - 1957

4 weeks ending	Zineb	Captan	Thiram	Control
13 October	10.2	8.9	11.8	4.4
10 November	30.9	26.5	30.2	24.4
3 December	19.5	17.5	16.9	13.3
5 January	6.2	6.3	6.9	4.4
2 February	2.4	5.5	4.4	2.7
2 March	9.5	8.9	5.5	0.4
30 March	55.3	37.3	24.4	13.3
27 April	26.5	20.0	15.5	7.5
4 May	54.0	37.8	39.1	16.4
Totals	214.5	168.7	154.7	86.8

1957 - 1958

4 weeks ending	Zineb	Captan	Thiram	Control
29 September	3.8	3.5	3.4	3.1
26 October	22.9	31.1	26.7	26.2
23 November	43.4	44.3	49.3	38.7
21 December	24.9	28.7	24.4	26.7
18 January	53.3	68.9	52.3	60.8
15 February	38.4	63.1	40.9	19.5
15 March	53.8	114.7	62.9	14.7
12 April	9.3	20.0	6.9	0.4
Totals	249.8	374.3	266.8	190.1

1958 - 1959

4 weeks ending	Zineb	Captan	Thiram	Control
20 September	34.0	37.5	18.0	25.3
18 October	85.0	95.5	62.8	80.0
15 November	90.8	107.0	72.0	91.8
13 December	24.5	36.3	30.8	32.0
10 January	20.3	30.0	17.3	24.0
7 February	10.3	20.5	10.3	10.0
7 March	18.0	36.5	28.8	21.5
21 March	7.3	27.8	14.3	10.3
Totals	290.2	391.1	254.3	294.9

1960 - 1961

4 weeks ending	Zineb	Captan	Thiram	Control
27 August	0.2	-	-	-
24 September	23.7	17.8	15.7	25.7
22 October	78.5	46.3	55.3	77.2
19 November	66.5	55.3	49.7	64.5
17 December	23.5	25.7	21.0	20.3
14 January	5.7	8.7	4.5	4.2
11 February	7.3	9.8	4.3	1.7
11 March	54.2	39.2	25.7	3.7
8 April	56.7	32.2	23.0	3.2
6 May	8.3	3.7	4.5	-
Totals	324.6	238.5	203.7	200.5

The figures underlined are those of particular interest as they indicate the greatest effects on yield due to the spraying treatments.

The table for 1959 - 1960 has been omitted, this season being a particularly bad one for the growth of Anemones and spraying with any fungicide was of little value.

1956 - 1957 Season

Spraying up to the end of December appeared to have no effects on plant health and gave no significant differences in yield. At the end of December a series of sharp frosts occurred the last of which was on 1 January. The ninth application of the spray treatments was applied on the following day under ideal conditions. The following day the weather changed completely with minimum night temperatures of 44° F. to 53° F and a relative humidity of 93% to 100%, ideal for the spread of Botrytis on frost damaged foliage. From this point differences between the treatments became apparent. Botrytis spread rapidly on the control plot and to a certain extent on the captan and thiram plots but zineb gave a clear indication of having maintained plant health to a fairly high degree. The effect on crop yields was not immediate but in March the number of flowers cropped was well above the control and better than captan and thiram.

1957 - 1958 Season

Here again beneficial results were obtained during the weeks following the new year but the results showed a surprising reversal in the effectiveness of zineb and captan, the latter giving by far the best results. General observations of the plots showed no reason for this as growth of the

Anemones had been reasonably uniform in both seasons. The answer could only be found by repeating the trials. One thing however, was confirmed, the necessity of spraying during the winter months after bad weather.

1958 - 1959 Season

The results of this season were similar to those of 1957 - 1958 with captan again giving the best results. The differences did not show until nearer the end of January when frost had damaged plants. Zineb was again disappointing in fact it appeared less effective than thiram.

1960 - 1961 Season

Rainfall was frequent during summer and autumn which made for good regular growth of plants and also for rapid secondary spread of Peronospora. Visual effects of all sprays were showing by mid-November there being more Botrytis infection on the control plot. A series of ground frosts occurring in mid-December followed by mild humid weather, similar conditions to those in 1956 - 1957, caused further differences to show between the treatments. In this case zineb repeated the success it had given in the first year's trials, with captan not nearly so effective. Attention was then drawn to other similarities between these two seasons. It was known that there was a high incidence of Peronospora infection in the summer and autumn of 1960, and research into records of another trial dealing specifically with Peronospora also showed that the disease had spread rapidly in 1956, but not to such an extent in the other two years. The probable explanation of the success of zineb in these circumstances is that this fungicide had been more specific in controlling Peronospora, thus giving the plants more resistance to adverse weather conditions, particularly frost.

Other fungicides

Early trials include a number of other fungicides which for various reasons were discontinued in further trials. Those which must be mentioned as causing severe damage by leaf scorch are all compounds containing copper in whatever form. This may be partly due to interaction with the salt in the atmosphere of the South West maritime climate, a point which must be considered whenever damage of this kind occurs. Other fungicides of little effect were tecnazine dust and some antibiotic sprays containing streptomycin. In general spray formulations of fungicides are far more effective than dust formulations of the same compounds, captan must be mentioned in this respect.

Of the newer materials tried recently the spray form of dicloran has given promising results; almost comparable with captan in a good captan year. Trials at Ellbridge sub-station have also shown maneb as promising.

Discussion

The varied results given by different fungicides indicate that control of Botrytis in Anemones is not by any means straight forward. The first conclusion drawn in the early years was that spraying was necessary

after mechanical damage due mainly to weather conditions. It did not at that time appear necessary to spray against Botrytis during the summer and autumn, and captan applied at the correct time would give the best results. But there were these two seasons when captan had failed which made this procedure questionable. It seemed that there was some factor involved which nullified the effect of a direct Botrytis controlling spray. From the results obtained with zineb it then became fairly clear that severe infection by Peronospora had an effect on plant stamina not always obvious during summer and autumn. If this disease was not controlled the chances of efficient control of Botrytis later in the season were considerably reduced. These trials have led to a revision of the general spraying programme at Rosewarne. Spraying with zineb is carried out regularly during August and September until the aerial spread of Peronospora is no longer apparent. An occasional captan spray is applied at this time if it is thought necessary and during the autumn and winter months this material is used regularly in place of zineb.

THE FUNGITOXIC EFFECTS OF DICLORAN ON BOTRYTIS CINEREA

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Summary

Dicloran (2,6-dichloro-4-nitroaniline) is fungistatic to the mycelium and spores of Botrytis cinerea. It delays germination and causes a severe check to hyphal growth. In certain media, dicloran causes distortion and bursting of the germ tubes. The nucleic acid level of B. cinerea mycelium increases in the presence of dicloran; the most marked increase occurs at low dosages (1 p.p.m.) when growth is checked without morphological abnormality. It is suggested that dicloran is a structurally non-specific toxicant exerting its effect by disorganising cell growth and division.

Introduction

For many years, Botrytis cinerea, the fungus causing grey mould on glasshouse lettuce crops has been controlled by the use of chlorinated nitrobenzene compounds. More recently, however, 2,6-dichloro-4-nitroaniline (dicloran) has also found commercial application for the control of this disease.

Laboratory and glasshouse experiments have indicated that dicloran and the chlorinated nitrobenzenes possess a number of common features and that they are active against a similar range of fungi. Thus, experiments on the mode of action of dicloran against B. cinerea have been designed in the light of results available from earlier work with the chlorinated nitrobenzenes.

Clark, Hams, Higgons and Stevenson (1960) have shown that, although dicloran has negligible activity in the slide germination test (American Phytopathological Society, 1943), its high toxicity to B. cinerea can be demonstrated by an agar impregnation test (Brook, 1952). The activity of tecnazine against this fungus may also be demonstrated by the impregnation test but tecnazine resembles dicloran in being ineffective in preventing spore germination on glass slides. Differential activity against spore germination and hyphal growth of Monilia fructicola has been described for a range of compounds by Horsfall and Rich (1953) and it has been suggested (Horsfall, 1956) that since spore germination (unlike hyphal growth) is independent of nuclear division, these compounds may exert their effects by checking mitosis. Horsfall attributes the inhibition of sporulation by the chlorinated nitrobenzenes to their effect on mitosis and some support for this suggestion is provided by the work of Carey and McDonough (1943) who showed that p-dichlorobenzene causes polyploidy in onion root cells.

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A series of laboratory experiments were therefore undertaken to compare the general effects of dicloran and tecnazine on spore germination and mycelial development in B. cinerea. An investigation was also begun into the effects of sub-lethal doses of dicloran on nucleic acid synthesis in the cells of this organism.

Materials and Methods

Fungicides

Phenylmercuric chloride (PMC) and phenylmercuric nitrate were used as the finely ground technical materials. These compounds were added to the medium in 0.5 ml. sterile distilled water, the concentration of the suspension being adjusted to give the correct final concentration upon dilution.

2,6-dichloro-4-nitroaniline and 2,3,5,6-tetrachloronitrobenzene were obtained by re-crystallisation of the technical material. These compounds were added to the medium in 0.5 ml. acetone, the concentration of the acetone solution being adjusted to give the correct final concentration upon dilution.

Fungus

A sporulating strain of Botrytis cinerea was used for all the experimental work. Cultures were isolated from natural infections on lettuce plants. Stock cultures were maintained on potato extract agar (200 mg/litre decoction). Spore suspensions were prepared by rinsing the surface of the cultures with sterile distilled water. For most purposes the inoculum was adjusted to give a final concentration of 50,000 spores per ml. Where mycelium was used as inoculum, uniform discs (7.5 mm. diam.) of malt agar bearing non-sporing mycelium from a 3-day-old culture were cut from a petri dish and placed in the culture medium.

Media

Malt extract, prepared by diluting malt extract with water to give a 2% solution.

Czapek, prepared as follows: sucrose (3.0%), sodium nitrate (0.2%), potassium chloride (0.05%), potassium phosphate (0.1%), magnesium sulphate (0.05%), ferrous sulphate (0.001%). Normally this medium was maintained at pH 4.0 using McIlvaine citrate-phosphate buffer, diluted with two parts of water.

Dextrose citrate, prepared as a solution containing 0.1% dextrose and 0.001% sodium citrate.

The above media were sterilised by autoclaving for 20 minutes at 20 lb./sq. in. Agar (2%) was used to solidify media where necessary.

Culture Methods

Cultures were grown in liquid media using bottles (vol. 350 ml.) outside dimensions 14.5 x 4.5 cm. placed horizontally with the largest side downwards. Petri dishes were used for experiments using solid media. Slide cultures were prepared by the method described by Knaysi (1957) and spore germination tests were carried out by the technique recommended by the American Phytopathological Society (1943). In all cases the organism was incubated at 23° C.

Assessment Methods

Mycelium grown in liquid cultures was separated from the medium, washed with distilled water and then dried to constant weight at 50° C. For more critical work the washed mycelium was freeze-dried before weighing. Linear growth on solid media was measured along two diameters of the dish at right-angles. In all experiments at least five replicates were used for each treatment. Germtube development in slide cultures was estimated by a grading system based on the number of septa evident at the different stages of development.

Dosage response data was analysed by the probit method and toxicity was expressed as the dosage required to reduce germination or mycelial growth by 50% (E.D.50 value). Saturation response data were treated in a similar way to obtain the chemical potential or R.S.50 value for different fungicides (Byrde and Woodcock, 1959). Saturated solutions were prepared by adding an excess of the finely-ground fungicide to sterilised medium and placing the flasks on a mechanical shaker at room temperature. The media were allowed to stand overnight and the clear supernatant was decanted off into sterile flasks. The saturated solution was then added to culture bottles containing the appropriate volume of normal medium to give a series of dilutions. Normally a dilution interval of one half was used, starting from an initial relative saturation of 0.25.

Determination of nucleic acids

The preliminary extractions of acid-soluble-phosphorus and lipid-phosphorus were carried out by the methods of Smillie and Krotkov (1960). The residue from these preliminary extractions was then dried, powdered and extracted three times with 2 ml. of 0.5 N perchloric acid at 70° C. Each extraction was continued for 30 minutes, the supernatants being combined. The total nucleic acid content of the sample was determined by measuring the extinction of the extract at 260 mμ.

Replicate comparisons were made on freeze-dried mycelium of B. cinerea to establish the reliability of the technique. Repeated determinations made on the same samples of mycelium indicated that an error of not more than 10% could be expected in the values for total nucleic acid obtained by this method.

Experimental

The first series of experiments were carried out to examine the general effects of a range of concentrations of dicloran on spore germination and mycelial growth in a variety of solid and liquid media. These were followed by experiments designed to study the specificity and reversibility of the toxic action of dicloran.

Solid media

On malt extract agar, a concentration of 1.07 p.p.m. dicloran was required to suppress mycelial growth of B. cinerea by 50%. When Czapek agar was used to culture the fungus, 1.45 p.p.m. dicloran was needed to obtain the same degree of inhibition.

Spore germination and subsequent mycelial development of B. cinerea on agar impregnated with varying concentrations of dicloran and tecnazine was examined under normal and phase-contrast microscopes. Slide-cultures were set up in which the agar was impregnated with 5, 25, 100 or 500 p.p.m. of either dicloran or tecnazine. Untreated agar was also inoculated with spores and served as control.

Even at the highest concentrations of dicloran and tecnazine the rate of germination and subsequent hyphal growth was only reduced to about a third of the rate recorded on untreated agar and eventually sporing colonies were produced. No morphological effects were evident under these conditions, the frequencies of septa and lateral branches being no different from the control. No spore germination was recorded in similar slide-cultures impregnated with 10 p.p.m. PMC.

In a further experiment, slide cultures were prepared from a small portion of the same impregnated agar as that used for plate cultures. The slide and plate cultures were then inoculated with spores from the same culture and, whenever observations were made on the slide cultures, discs of agar bearing the spores and developing colonies were taken from the plates and also examined. In a comparison of the effects of 10 p.p.m. PMC and 10 p.p.m. dicloran on germination of spores of B. cinerea in the two types of culture, no germination was observed within 8 days in the presence of PMC, in either the plate or slide cultures. Furthermore, no germination occurred during this period in plate cultures impregnated with dicloran although slow growth occurred in the slide cultures and the mycelium began to sporulate by the fifth day after inoculation.

Liquid media

The toxicity of dicloran to mycelium of B. cinerea in liquid culture was examined in accordance with Ferguson's Principle (Ferguson, 1939), where the inadequacy of expressing toxicity in terms of applied concentration is overcome by using the chemical potential (or thermodynamic activity) of the fungitoxic compound. The chemical potential of toxicants, applied in solution, may be expressed as S_t/S_o where S_t is the molecular concentration of the toxic solution and S_o its solubility in mol./litre. Thus the concentration required for 50% inhibition of mycelial growth was express-

ed as relative saturation (R.S.50) rather than dosage (E.D.50). Parallel tests were carried out with PMC. The R.S.50 value of dicloran against B. cinerea in Czapek medium was found to be 0.21; that of PMC was 0.0004 under the same conditions.

Spores of B. cinerea germinated within a few hours when added in droplets of nutrient solution on to clean glass slides. In the presence of dicloran, no growth check was visible until the fungistatic concentration exceeded 8 p.p.m. As with solid media in slide cultures, concentrations of dicloran up to 500 p.p.m. merely caused a retardation in growth rate and no gross changes in morphology occurred. Reducing the concentration of spores in each droplet from 100,000/ml. to 80/ml. did not significantly increase the fungitoxicity of a given quantity of dicloran.

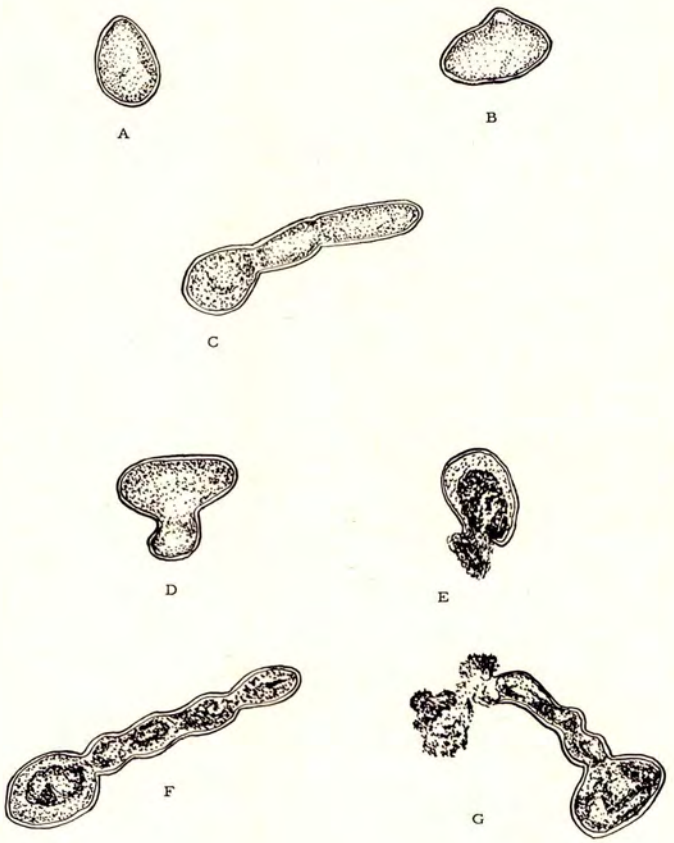
Dicloran produced a marked effect on hyphal growth when introduced into flask or bottle cultures. With most natural isolates of B. cinerea, germination was almost completely inhibited at 5 p.p.m. or above and at lower concentrations very severe distortion of the germ tubes was caused. Thus spores produced short germ tubes which became constricted at intervals along their lengths and often swollen at their tips. Frequently the germ tube would burst allowing the protoplast to stream out from the ruptured tip. The effects are illustrated in Fig. 1. The optimum concentration for the more severe morphological effects lay between 1 and 5 p.p.m. but also occurred at slightly higher concentrations with some isolates. Below a concentration of 1 p.p.m. the germ tubes showed less tendency to burst and only slight malformation. The growth rate was, however, still checked under these conditions.

When Czapek medium was replaced by dextrose citrate medium for germinating spores in bottle cultures, no bursting of the hyphae occurred in the presence of dicloran at 25, 5 or 1 p.p.m. Germination was very slow in this medium but, after 5 days, germ tubes were produced in cultures containing 5 and 1 p.p.m. dicloran. The germ tubes showed none of the abnormalities associated with Czapek medium. On the other hand, in similar experiments using a liquid 2% malt extract medium, deformed hyphae and burst germ tubes were again produced in the presence of 10 and 2 p.p.m. dicloran.

Reversibility of Toxicity

The term "fungicidal" is normally applied to a fungitoxic compound which penetrates to the site of action within the protoplast where it causes irreversible damage to the metabolism of the cell. On the other hand a "fungistat" effects its toxicity only while an adequate concentration of the substance is maintained at the site of action and thus fungistatic effects are normally reversible, once the fungitoxic concentration is reduced.

In a preliminary experiment to examine this point, discs of mycelium were grown in liquid Czapek medium containing 10 p.p.m. of phenylmercuric nitrate (PMN), tecnazine or dicloran. A further set of discs were grown in media containing no added fungicide. No growth occurred in any of the treated cultures but, after 10 days, the discs were taken from the fungitoxic solutions, washed in sterile water and then transferred



A - C. Control series in normal Czapek medium.
D - G. Treated series in Czapek medium containing 5 p.p.m. dicloran. A. B. cinerea spore before germination.
B. 6 hours after germination. C. 18 hours after germination.
D. 18 hours after germination in treated medium.
E, F and G. 48 hours after germination in treated medium showing deformed and burst germ tubes exuding contents from hyphal tip (x 2,500).

Fig. 1

aseptically to fresh culture bottles containing no added fungicide and incubated for a further 10 days. At the end of this period discs receiving pre-treatment with either tecnazine or dicloran were growing normally and at the same rate as the mycelium in bottles inoculated with discs receiving no pre-treatment. However, discs receiving pre-treatment with PMN showed no signs of growth and had evidently been killed by this fungicide.

A quantitative experiment was then carried out to determine whether full recovery occurred or whether dicloran left a residual effect. Discs of mycelium were held in liquid cultures containing 50 p.p.m. dicloran for periods varying from 10 - 70 days. After the given period, the discs were washed and transferred to culture flasks containing no added fungicides and incubated. 10 days later the dry weight of mycelium was determined and compared with the dry weight of mycelium produced by discs of the same culture strain which had received no pre-treatment with dicloran. The results (expressed in Table 1 as the percentage reduction in dry weight) suggest that recovery is virtually complete when the fungus is transferred within 2 or 3 weeks to media containing no added fungicide. However, when held in the presence of the toxicant for over a month, recovery may be slightly delayed.

Table 1 The effect of period of inhibition by dicloran on rate of recovery of mycelial discs of B. cinerea

Period of inhibition (days)	10	20	30	50	70
Reduction in dry weight (percentage)	0	5	10	26	35

The fungistatic nature of the toxicity of dicloran was again demonstrated by incubating spores of B. cinerea in dextrose-citrate liquid cultures in the presence of 25, 5 and 1 p.p.m. dicloran. After 15 minues, 18 hours and 43 hours, samples of the spores were centrifuged down, washed in sterile distilled water and then resuspended in medium containing no added fungicide. In all instances germination took place within 24 hours of transfer and after pre-treatment at 5 and 1 p.p.m., the growth rate in untreated media was normal. Some check in the growth rate was, however, noted during the first 24 hours following pre-treatment for 18 or 43 hours with 25 p.p.m. dicloran.

The effects of dicloran on nucleic acid synthesis

The effects of decreasing concentrations of dicloran on the nucleic acid level in mycelium of B. cinerea were examined. Spores were germinated in 5, 2.5 and 1.25 p.p.m. of dicloran and incubated in liquid Czapek medium contained in culture bottles for 48 hours. Two sets of untreated cultures were grown under the same conditions and harvested after 24 and 48 hours. Slide preparations were made from small samples of the different cultures at harvest. Spores germinating in the presence of 5 p.p.m. dicloran showed bursting at an early stage and only about half

the spores successfully produced germ tubes, most of which were severely stunted and deformed. In 2.5 p.p.m. dicloran, germ tubes were again malformed but the majority of the spores had germinated without bursting. In 1.25 p.p.m. dicloran the germ tubes were only slightly distorted but were less than half the length of the germ tubes of the 24-hour control cultures. Table 2 shows the total nucleic acid in the treated cultures expressed as a percentage of the freeze-dried weight of the mycelium. The total nucleic acid in untreated mycelium at a similar stage (24 hours) of growth and after a similar period of time (48 hours) to the treated cultures is also given.

Table 2 Effect of different concentrations of dicloran on total nucleic acid in B. cinerea mycelium

Age of culture (hours)	Treatment	Total nucleic acid (percentage of dry weight)
24	Untreated	1.48
48	Untreated	1.08
48	Dicloran 5.0 p.p.m.	1.34
48	Dicloran 2.5 p.p.m.	2.01
48	Dicloran 1.25 p.p.m.	3.45

The results given above suggest that total nucleic acid in B. cinerea cells is considerably increased by the presence of sub-lethal amounts of dicloran. The highest increase is associated with a relatively low dicloran concentration of 1.25 p.p.m. Presumably this concentration permits RNA synthesis but slows up cell growth. At higher concentrations more drastic mechanical effects are produced, so that any trend towards increasing nucleic acid content is offset by the larger proportion of spores which fail to produce intact germ tubes and therefore cease to synthesise nucleic acids.

The total nucleic acid level was shown to vary in untreated cultures of increasing age and, to avoid variations which might be due to age rather than to the presence of dicloran, the compound was added 6 hours after the start of the experiment to a proportion of the culture bottles, when germ tubes were just beginning to emerge from the spores. The cultures were harvested after 48 and 96 hours. The results are given in Table 3.

Table 3 Effect of dicloran (added after 6 hours) on total nucleic acid in B. cinerea mycelium

Age of culture (hours)	Total Nucleic Acid (% of dry weight)	
	Untreated	Dicloran (2.5 p.p.m.)
48	1.15	2.95
96	1.62	3.21

Initial estimates of the nucleic acid level in mycelium harvested after 24 hours failed to show significant differences between the dicloran-treated and untreated cultures. However, by 48 hours the total nucleic acid level in the dicloran-treated cultures had more than doubled, and by 96 hours the total nucleic acid in the treated cultures was considerably in excess of that of the untreated cultures at any stage of their growth.

Discussion

The spore germination experiments illustrate a marked reduction in the activity of dicloran when tested on microscope slides rather than in plate or bottle cultures. The same discrepancies were recorded in both liquid and solid media. It is evident, however, from the high R.S.50 value that a relatively high concentration of dicloran must be maintained in the fungal cell to effect maximum activity and it is possible that availability of the toxicant may have been limiting the activity of dicloran on glass slides. These results illustrate the limitations of the slide germination tests in the evaluation of certain groups of funtitoxic compounds.

The results of the experiments described above indicate that dicloran is fungistatic towards mycelium and spores of B. cinerea. In this respect it resembles tecnazine and differs markedly from PMN which is a true fungicide causing irreversible toxicity. However, there is evidence (Table 1) that prolonged exposure to dicloran in liquid culture causes some residual inhibition after the fungistat has been removed from the medium. This may simply reflect the occurrence of a longer lag-phase where the period of inhibition has been relatively long, since the data given in Table 1 are based on the dry weight of mycelium produced in the 10-day period following transfer.

The reversibility of the toxicity of dicloran, together with its relatively high thermodynamic activity suggest that its mode of action is structurally non-specific. It should be noted, however, that an R S.50 value over 0.1 may, in some cases, be due to the detoxification of the fungicide and is therefore not an invariable guide to non-specific toxicity.

Although dicloran does not prevent the germination of B. cinerea spores, it does delay the appearance of the germ tubes and causes a severe check to hyphal growth. Under certain conditions it may also cause distortion and bursting of the germ tubes. Other fungitoxic substances, such as α-naphthylamine and camphor (Bauch, 1941) and actidione (Wallen et al. 1950) produce similar, abnormal fungal cells while Priest (1960) has recently reported that the dichloronitrobenzenes produce severely misshapen hyphae in B. allii. Since differential activity towards spore germination and hyphal growth may be indicative of a toxic mechanism preventing cell division, and hyphal distortions have been found in association with mitotic aberrations caused by a range of other fungicides (Horsfall, 1956), it is probable that dicloran disorganises the processes involving cell division.

It is evident from Tables 2 and 3 that this disorganisation results in a considerable increase in the nucleic acid level of the cells. From other

data, not quoted here, it was apparent that about 80 - 85% of the total nucleic acid content of the extracts represents RNA and thus, there must be a significant increase in the RNA of the treated cells. Since the nucleic acids are responsible for cell reproduction and protein synthesis the increase recorded here, supports the view that dicloran is effecting its toxicity by disorganising cell growth and division.

It is not yet clear how the increase in RNA is caused but, in view of the relative ease with which aniline dyes form complexes with polynucleotides, it is possible that dicloran may form a weakly bound complex with the cytoplasmic nucleic acid and thus block protein synthesis. The reversible nature of dye-polynucleotide complexes (Steiner and Beers, 1958) would be in keeping with a non-specific, fungistatic mode of action.

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