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GENETIC CONTROL OF THE ONION-FLY, HYLEMYA ANTIQUA, IN THE NETHERLANDS

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Summary. The development of a sterile male project is characterized by four consecutive phases: 1. laboratory research 2. small scale field testing 3. technical development and large scale field testing 4. application.

The onion fly project in the Netherlands is currently on the verge of phase three. Results from three small field trials indicate the suitability of the sterile male approach for onion fly control. Further technical development is necessary to increase the scale of operations and to meet the economic demands.

Résumé. Le développement d'un projet de "mâles steriles" est caracterisé par quatre phases consecutives: 1. recherche en laboratoire 2. essais en champs en petite échelle 3. développement technique et essais en champs en grande échelle 4. application pratique.

Aux Pays Bas le projet "mâles steriles" contre la mouche de l'oignon est sur le seuil d'entrer dans la troisième phase. Les résultats de trois essais en champs sur 1 ha. indiquent qu'en principe la lutte génétique peut être utilisée contre la mouche de l'oignon.
L'amplification de l'échelle des opérations nécessite un développement technique continué en tenant compte des réalités économiques c'est à dire visant à un prix de revient compétitif.

INTRODUCTION

Since 1965, research has been carried out on the possibilities of controlling the onion fly, Hylemya antiqua, by means of the "sterile male" technique. At that time the problems arising from resistance of the fly to some organochlorine insecticides demanded attention. Measures taken to ensure the future control of the fly included the establishment of a program to study the feasibility of the "sterile male" technique as a possible control method.

Currently many similar projects are in progress with a few in the stage of commercial application (Lindquist, 1973). An important role is played by the joint F.A.O./I.A.E.A. division of Atomic Energy in Food and Agriculture which stimulates research and development in this field. The International Organization for Biological Control is also actively engaged in genetical control.

The principles of the "sterile male" technique are simple and generally known (LaChance, 1967). However, an appropriate research programme is necessary to be able to decide whether or not in a specific case the method can be applied. In such a programme confirmation of laboratory data by experiments under field conditions must necessarily be included. The onion fly project in the Netherlands is still in a stage of biological testing. In the near future, however, technical and economical factors will determine whether this control will be suitable for development into a regular control method for onion-growers in the Netherlands and elsewhere.

PHASES

The work has been divided into 4 main phases each of which has its own characteristics and demands.

1. laboratory research
2. small scale field testing
3. technical development and large scale field testing
4. application

Phase 1.

Preliminary research has been carried out to decide whether a number of basic conditions could be met. Previous information has shown that many programmes similar to the onion fly project became failures for various reasons. Most of these reasons originated from lack of knowledge on basic problems connected with the method. Lindquist (1973) recently summarized the requirements which must be fulfilled in order to be able to apply the "sterile male" technique with a fair chance of success. The relative importance of these requirements is determined by the biological properties of the insects involved. One of the characteristics of the method is the specificity, the efforts and effects being restricted to just one species only. Therefore, all research, methods and use of materials must be tailored to the specific possibilities and problems of the species concerned. Consequently, detailed information to enable solution of problems must be acquired for the species to be controlled. Experience with and knowledge from "sterile male" projects on other species can have a restricted value, but, nevertheless, can be invaluable as a check.

Key problems, however, are associated with the possibility of an economically feasible mass-rearing on an artificial diet, the possibility of sterilizing the insects effectively without impairing a normal functioning of its physiology and behaviour and sufficient knowledge of its population dynamics in the field. The diversity of these problems requires a team approach to study these various aspects.

Phase 2.

The results of phase 1 determine whether the project should be continued. This phase of laboratory research can be of relatively short duration when problems are encountered which prohibit further progress. The second phase of small scale field trials requires about 4 years to develop and modify methods and to acquire experience in the field. By a coherent series of small field experiments the scientific feasibility of the "sterile male" approach must be demonstrated. During this phase already attention should be given to economical factors in view of future demands of economical efficiency.

Phase 3.

When small scale field trials demonstrate scientifically the possibility to use the "sterile male" method against a particular insect, the project enters the next phase. In the third phase it is necessary to emphasize technological and economical factors required to develop mass-rearing and other methods into an economically competitive and technically practical method of control. Biological research is still needed to provide background information and solutions of problems caused by the process of enlargement and de-

velopment. The main points in this phase of development are the change from rearing on a laboratory scale to mass-rearing and the adaptation of release and evaluation methods appropriate to the size of the area to be treated. Large scale field trials must prove the economical and technical utility of the control method.

Continuation of the research work and small scale field testing into a technical and economical development require timely support by governmental authorities, growers associations and industrial or commercial organizations.

During this period the team tends to shift from research oriented entomologists to people with a more industrial approach. This may cause problems of technical and personal nature which may be prevented by a timely anticipation.

Phase 4.

Once the effects of the control method on a large scale have been successfully demonstrated the final stage has been reached. Practical application, however, needs preparation for it to be effective over longer periods. Measures should be taken in advance to assure a maximal effectiveness. Insect control in this way is not an individual decision of the grower which can be changed at any time. In addition, the application will start in a specified area which will be expanded gradually as a satisfactory degree of control is achieved. Such a well prepared campaign should be so that all concerned cooperate fully for their mutual benefit.

RESULTS

After preliminary investigations (de Fluiter et al., 1967, Ticheler and Noordink, 1968), laboratory research has been organized as two consecutive 3-year plans. Research topics have included: nutritional and other properties of the larval medium (Ticheler, 1971), pupation, production and storage of pupae, egg production and rearing efficiency (Noorlander, unpublished); irradiation and competitiveness of irradiated flies (Noordink, 1971), histopathological effects of irradiation (Theunissen, 1971), spermatogenesis and oogenesis (Theunissen, 1973 and 1974), cytogenetics (van Heemert, 1973, Wijnands-Stäb and Van Heemert, 1973), marking, trapping methods, dispersal and population dynamics (loosjes, unpublished), and computer simulation models (Wijnands-Stäb and Frissel, 1973; Loosjes and Frissel, unpublished; Frissel and Wijnands-Stäb, 1973). The aims set forth in the 3-year plans have been achieved over this time and as a logical consequence small scale field testing has been carried out during 1971, 1972 and 1973 as a part of a 4-year programme. The field experiments are being done on a number of adjacent 1 ha fields well away from commercial onion-growing areas. In 1970, a population was established in the experimental area by releasing fertile onion flies. The resulting population of overwintering pupae has been estimated at 20 000, about the order of magnitude of numbers of onion flies per ha developing in onion-growing areas subjected to insecticide treatment. For three years this population has now been controlled solely by releasing sterilized flies. Details of the releases and the methods concerned have partly been published (Ticheler et al., 1972).

Important features of the releases have been checked by means of a number of independent criteria:

1. The percentage of emergence of sterilized flies from irradiated pupae.
2. The number of trapped flies; of these flies we determined:
   a. the sex-ratio.

b. the sterile/fertile ratio in males and females.

c. the percentage mated sterile and fertile females.

d. the rate of Entomophthora spp. fungus infestation in both sterile and fertile females.

3. The sterile/fertile ratio of the eggs laid by the trapped fertile females.

4. The number of infested plants.

5. The number of overwintering pupae.

The results of the releases in terms of population control show the following sequence:

1970:	20 000	overwintering pupae
1971:	17 000	" "
1972:	5 000	" "
1973:	400	" "

The percentage of sterile flies in the trapped population in the weeks during the field experiments is summarized in Table 1.

In a declining population of fertile females the number of egg batches, which can be used to determine the egg sterility, also decreased. Particularly in the late spring and early summer, egg hatch data can show considerable variability due to low numbers of egg batches. The weekly average of egg sterility is taken as a criterion (Table 2).

Damage to the crop has been low in the experimental plots (Table 3). Control plots in the experimental field during the first two years did not function due to habitat requirements of the fly which were unknown at that time.

DISCUSSION

Apart from the decrease in the overwintering populations of onion fly pupae, effects of the releases of sterile insects can be evaluated from the percentage of sterile flies and eggs. Interpretation of these data from the 1971 and 1972 trials have already been given (Ticheler et al., 1972). In summary, a satisfactory similarity between the percentage of sterile flies in the population and the percentage of sterile eggs, laid by fertile females, has been found. This indicates a normal competitiveness of the sterilized flies in the mixed population. When some factor interferes in the normal performance of the sterilized flies a discrepancy occurs between both these factors, demonstrated by a drop in the percentage of sterile eggs. This happened for instance during the second flight in 1971, when the sterile flies seemed to be preferentially attacked by an Entomophthora-fungus due to the release method. After modification of this method no preferential infection could be found.

Due to both a high number of released sterile flies in 1973 and a low wild population, estimated at 5 000 flies at the most, ther percentage of sterile flies in the population was high (Table 1). A very low number of fertile females were trapped during May and June. Accordingly, egg production by these flies in the laboratory has been low in absolute numbers.

During previous field trials releases over the period of the first flight of the wild population took place in the field, cropped in the previous year

with onions, in order to permit the emerging fertile flies to mix immediately with sterilized ones. Troughout 1973, the sterilized flies were released in the onion field itself to determine whether this would give satisfactory results in view of an increased efficiency for practical application. The relatively low percentage of sterile eggs in May and June may indicate that too many females arrived fertilized at the onion field. When flies remain for some time in the area in which they emerged, they are already mated when they reach the cover of the sterile population, in particular, because the density of this population decreases rapidly outside the onion field. The first mating, being generally the most important one, determines the percentage of egg sterility. Another factor in the generally low level of egg sterility during the field trial is the possibility that the population of fertile flies in the immediate surroundings of the onion field has reached such a low level that these flies can be effectively dealt with, but fertilized females immigrating into the area cause low egg sterility although the absolute figures are low. The relative absence of local fertile females results in a low egg production, eggs may be produced by immigrating fertilized females. When larger areas are treated this factor should have a relatively decreasing significance.

Results from three years of field trials allow the following general conclusions to be made: the performance of the sterilized flies in the field is comparable to that of the fertile flies, in proportion to the relative numbers, demonstrated in egg sterility. Care must be taken to apply suitable release methods to prevent unnecessary disadvantages for the sterile flies. Releases must take place where fertile flies are expected to emerge and measures must be taken to prevent predation by mice and birds. Damage to our crop has always been at a level below that unacceptable to the farmer. An onion fly population which is not controlled at all tends build up quickly. The decline of populations of overwintering pupae also suggest a satisfactory control by the sterile flies.

PROSPECTS

During the first two phases of the onion fly project the economic implications of the development of techniques and methods have always been kept in mind. They did influence decisions as to which line of research or development should be followed. The picture of the economic reality and the requirements of maximal efficiency have always been implied in the activities during the two phases which are presently almost terminated.

Efficiency is served by a smooth transition from phase 2 to phase 3. Before the first field trial was carried out we made an internal study to anticipate this transition, on the assumption that the results of phase 2 would be satisfactory. Based on our knowledge from mass-rearing and field techniques at that time estimates were made of the costs of mass-rearing and of release, supervision and evaluation. A timetable and research plan for a gradual transition period was made. In the light of recent data costs are much lower than had been expected, owing to more efficient rearing methods and field techniques. However, important developments have still to be achieved during the third phase in order to obtain a truly competitive method for practical application.

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Table 1. Weekly percentage of sterile flies during field trials.

Month	Week	1971	1972	1973
May	1	-	50.0	-
	2	52.8	58.6	99.4
	3	75.0	83.3	99.2
	4	84.0	83.7	95.9
	5	84.0	86.9	88.6
June	1	70.1	88.3	80.8
	2	66.7	80.9	87.3
	3	67.1	88.0	94.3
	4	38.0	47.9	92.6
July	1	28.8	50.7	92.4
	2	60.2	29.6	94.3
	3	60.2	72.0	93.5
	4	63.0	80.6	81.8
August	1	52.9	88.1	69.1
	2	38.9	78.4	92.0
	3	21.0	71.0	92.5
	4	34.3	44.9	96.1
	5	-	52.2	-
September	1	-	51.2	99.2
	2	-	70.1	-
Mean		56.0	67.8	91.1

Table 2. Weekly percentage of sterile eggs and total number of eggs during field trials.

Month	Week	1971		1972		1973	
		%	number	%	number	%	number
May	1	-	-	-	-	-	-
	2	61.8	1318	97.8	96	-	-
	3	65.3	101	100	11	-	-
	4	78.5	751	0	14	43.3	90
	5	75.1	410	26.9	93	100	95
June	1	80.8	994	100	50	32.3	136
	2	50.3	660	90.6	138	18.2	176
	3	56.5	976	9.0	44	52.7	148
	4	30.9	534	49.6	661	16.4	110
July	1	12.3	439	45.7	1562	41.5	183
	2	11.1	637	36.3	1171	16.9	65
	3	15.1	1779	53.3	1156	81.7	580
	4	13.4	666	46.9	1696	81.5	702
August	1	22.6	1342	86.4	273	69.4	271
	2	3.1	258	79.4	451	27.3	11
	3	17.4	218	68.7	969	82.8	99
	4	16.0	564	70.3	1202	100	31
	5	-	-	48.8	1167	-	-
September	1	-	-	49.2	1185	-	-
	2	-	-	41.8	201	-	-

Table 3.

Number of infested onion plants/ha *

	Experimental field	control plot
1971	52 200	-
1972	6 000	-
1973	1 200	11 200**

* average number of onion plants is about 10^6 plants/ha.

** control plot at some distance from experimental field infested with laboratory reared, fertile flies.

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THE EFFECTIVENESS OF INSECTICIDES APPLIED TO SOIL

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Summary Most of the factors determining the performance of an insecticide applied to soil are now probably recognised but the complex relationships between them are not well-understood. A basic structure for the relationships is proposed. Firstly, decision factors such as choice of insecticide, formulation, dose and application method determine the mean and distribution of concentration in the soil. A complex of indigenous factors associated with the soil, the insecticide and the climate then determine the persistence, the potential biological activity and, for each pest, the population reduction caused by the treatment. This is termed its efficiency. This efficiency combines with effects of natural enemies of the pest and with the intensity of the attack to determine the pest infestation surviving treatment and hence the loss of crop. Deducting the loss of crop from the potential yield leaves the marketable yield which is a measure of the ultimate effectiveness of the treatment.

Sommaire La plupart des facteurs qui déterminent l'action d'un insecticide appliqué au sol sont probablement reconnus, mais les rapports complexes entre eux ne sont pas bien compris. Cet article propose une analyse de la structure de base pour ces rapports. En premier lieu, des facteurs de décision tels que choix d'insecticide, leur formulation, les doses, et la méthode d'application, déterminent le moyen et la distribution de la concentration dans le sol. L'ensemble des facteurs indigènes se rapportant au sol, la nature de l'insecticide lui-même et le climat, déterminent ensuite la persistance des résidus, l'action biologique potentielle et, pour chaque insecte, la reduction de la population attribuée au traitement, c'est ce qu'on nomme l'éfficacité. Cette éfficacité alliée aux effets produits par les ennemis naturels de la perte ainsi qu'à l'intensité de l'infestation détermine le nombre qui survive au traitement et, par conséquent, le degré de perte de la recolte. En deduisant cette perte réelle de la recolte potentielle, on arrive à la recolte éffective et commerciale, la mesure du succés final remporté par le traitement.

INTRODUCTION

At the last Conference, Gair (1971) discussed the inadequacy of alternative insecticides for the persistent organochlorine compounds. Soil-inhabiting insects in particular were not being satisfactorily controlled and now, two years later, the position has not improved. There is dissatisfaction with the ability of many of the newer chemicals to protect crops to the standards required, especially those destined for processing. Despite many efforts, very few ways have been found to improve their performance and it is therefore opportune to consider possible reasons for their relatively moderate performances in soil. Fortunately, we now have some basic knowledge of the factors which affect the performance of insecticides in soil, and after more than 25 years of insecticide usage, we can recognise at least some of the

reasons for their limitations.

The practical uses of insecticides in soil have developed largely from ad hoc experimentation. Until the early 1960's, few organophosphorus insecticides had been included in experiments to control pests in the soil and knowledge of their residues and degradation was negligible. As problems began to arise with the organochlorine insecticides, from about 1963 in the U.K., attempts were made to determine the performance of likely alternatives, mainly organophosphorus compounds but including some carbamates. Unfortunately, infestations of some pests such as carrot fly were then so low that false conclusions were formed about the adequacy of the likely alternatives and, by 1968 when greater reliance had to be placed on them, it became apparent that they were usually inferior to the organochlorine insecticides against soil-inhabiting pests. This has been attributed to the shorter persistence of the newer compounds in soil compared with the stability of, for instance, dieldrin and DDT. However, this may not be the only reason for the present problems and, in any case, compounds very much more stable in soil than chlorfenvinphos, fonofos or phorate, for example, would probably not be acceptable today because of the risks of residue-contaminated crops and environmental side-effects.

What is limiting the performance of soil-applied insecticides? Can they be made to work more adequately in practice? Some of the important factors involved are discussed in this paper, referring particularly to problems associated with the control of pests of vegetables.

PEST CONTROL SYSTEMS

Since the hit-or-miss approach to improve the performance of soil-applied insecticides has been only partially successful in achieving satisfactory levels of pest control, a better understanding of the processes involved may enable further improvements to be sought more systematically. To do this, it is first necessary to identify, inter-relate and quantify the factors regulating the pest control systems, and therefore responsible for changes in status of pests.

Watt (1970) concluded from a consideration of available evidence that pest control systems are 'complex' in the terms of the systems analyst and that they are characteristically 'counterintuitive' in suggesting corrective actions which may often be ineffective, or produce opposite effects to those intended. Many factors interact through numerous feed-back loops so that cause and effect may be widely separated both in space and time. They may not therefore be obvious and so the selection of an appropriate control strategy is often difficult. Complex systems in general, however, seem to have many common properties so that results from studies in other subjects, such as urban dynamics, may be helpful in understanding difficulties with pest control. Watt cites three points made by Forrester (1969) arising from experiences in simulating corporations and city organisations. Firstly corrective action taken by organisations to overcome difficulties may make them worse, generating pressure for further action which only intensifies the problem. This is very reminiscent of many past attempts to improve levels of pest control by increasing insecticide usage, whereas a more judicious, limited use of insecticides may be more beneficial as Conway & Wood (1964) found in Malaysia. Secondly, although complex systems may resist large changes in the values of many variables, they can be very sensitive to changes in a few parameters or in the structure of the system. The parameters to which a system is sensitive are not usually self-evident but must be discovered by examining the dynamics of the system by simulation procedures. Thirdly, failure to understand systems is often blamed on the inadequacy of available data, whereas the real barriers may be deficiencies in the theories in the structure, the

inter-relations between the factors within the system. This point could be very relevant in the context of pesticides in soil. Much of the very large amount of data needed for dynamic modelling of the processes may already exist and a few new, critical experiments might fill the gaps in knowledge on some problems - if we knew where the gaps were, as Watt also points out.

To describe the performance of insecticides, it is essential to distinguish clearly between their efficiency in controlling the pest population and their ultimate effectiveness in aiding in the production of economic yields of pest- or damage-free crop commodities (Wheatley, 1974); this distinction was illustrated by a much-simplified description of part of the system involved in controlling carrot fly on carrots. The term efficiency was proposed to describe the ability of a control measure to reduce a pest population of given susceptibility by a definite proportion irrespective of the magnitude of that population. Efficiency is therefore, within limits, characteristically independent of the density of the pest population and can be expressed as a percentage reduction in pest numbers or damage. However, it may tell us little about the ultimate performance of an insecticidal treatment in influencing marketable yield - that is, about its effectiveness. This depends not only on the efficiency of the treatment but also on the relationships between many other factors such as the intensity of the pest infestation, associated natural enemies, the agronomic, chemical, seasonal and climatic factors determining crop growth, and the density of the host plant within a locality or a crop. Even when efficiency remains constant, effectiveness could be radically different if, for example, the level of infestation or plant density is altered. There is an important natural feed-back loop within this part of the system; the intensity of infestation influences the numbers of insects which survive a treatment, hence the infestation starting the next generation, and so on.

With soil-applied insecticides we are dealing with some of the general limitations of the insecticidal method of pest control, particularly in the factors linking efficiency to effectiveness as just described, as well as with limitations specifically imposed by the factors operating within the soil environment. These appear mainly to influence the part of the system determining the efficiency of a treatment and consequently 'soil' does not appear as a major variable in the relationships between efficiency and effectiveness illustrated for carrot fly control by Wheatley (1974; Fig. 2), although it must play a complicating role influencing effectiveness via crop growth. It is therefore apparent that, unless insecticidal efficiency is 100%, which cannot normally be achieved, attempts to correct the inadequacies of insecticidal control of soil-inhabiting pests solely by studying the influences of soil on efficiency are likely to be only partially successful, a point probably very relevant to present difficulties.

The role of soil and associated factors in determining insecticidal efficiency is now discussed, followed later by a consideration of some of the important relationships linking the efficiency of treatments to their effectiveness.

FACTORS INFLUENCING EFFICIENCY

Accepting that perfect (100%) efficiency is unattainable at present, some proportion of a pest population must survive any treatment. The percentage reduction in the pest population can only be assessed by determining the numbers of the pest present on comparable treated and untreated crop at some time after treatment, as in appropriately designed field trials. Efficiency, measured as the ability of the treatment to reduce a pest population, cannot usually be determined, therefore, merely by observing the performance of treatments in practice. Hence reports of erratic or poor performances on commercial crops should not be automatically interpreted as implying problems with efficiency in the present context, although this may often be the case.

The intrinsic potency of insecticides is not usually in doubt, unless there is reason to believe that the pest population has become resistant. By the time insecticides are marketed, substantial evidence should exist from field trials under a range of conditions in different seasons to indicate that they are capable of controlling the particular pests against which they are intended to be used. Unfortunately, there is no single accepted definition of what constitutes 'control', nor is there agreement on the degree of 'control' necessary for individual problems. By unequivocably defining the ability of a treatment to reduce a pest population, or its damage, the present concept of efficiency seems to provide an appropriate description of that part of 'control' for which the insecticide treatment is largely responsible. Invariable failure ought not to occur with approved methods, but problems do arise from inconsistent performance of treatments. Erratic performance can be caused, at least in part, by variations in efficiency, such as can arise from poor application, but this is not always so. Very consistent performance is now expected of treatments. In 1971, a meeting between research, advisory and industrial interests discussed reports of the failure of chlorfenvinphos granules to protect transplanted brassicas from cabbage root fly attack when they were applied in bands to the soil surface along the rows. The failure rate was estimated to be from 3 to 10%, implying success on 90 to 97% of the times that the treatment was used. This was considered to be sufficiently unsatisfactory to merit promoting the use of an alternative application method, sub-surface placement.

Results from experiments indicate that the efficiencies of insecticides applied to soil for cabbage root fly and carrot fly control do not vary widely under U.K. conditions if they are used in exactly the same way on all occasions. In other words each treatment tends to have a characteristic efficiency in comparable conditions. Some of the factors known to modify the efficiency of insecticides in soil are within the jurisdiction of the grower applying the treatments, referred to here as 'decision factors'. Others are indigenous to the soil or cropping systems, or to the pest population and all are subject to the physical influences of climatic factors. (Table 1).

Table 1

Relationships of factors influencing potential efficiency

Factors		Effects		
Decision				
Chemical, Dose, Formulation		Potency, Selectivity, Stability		
Application method		Volatilisation loss, Uniformity of dose, Concentration distribution	Persistence & Potential biological activity	Observed biological activity = POTENTIAL EFFICIENCY
Indigenous				
Soil, Climate		Soil state		
Pest susceptibility				

Decision factors

The factors which the grower himself imprints on the system are mainly associated with the choice of insecticide from a range of 'alternatives', the dose, and the method and time when it is applied. When recommendations offer a choice of chemicals,

they tend to imply that these are interchangeable alternatives, but this is often not so, some being more suited to certain conditions of usage than others. Recommendations are becoming more specific, for example in indicating that phorate is preferable to disulfoton or chlorfenvinphos for carrot fly control on fen soils. Many more constraints of this type will have to be fitted into recommendations in the future to ensure consistently high efficiencies under a wide range of conditions.

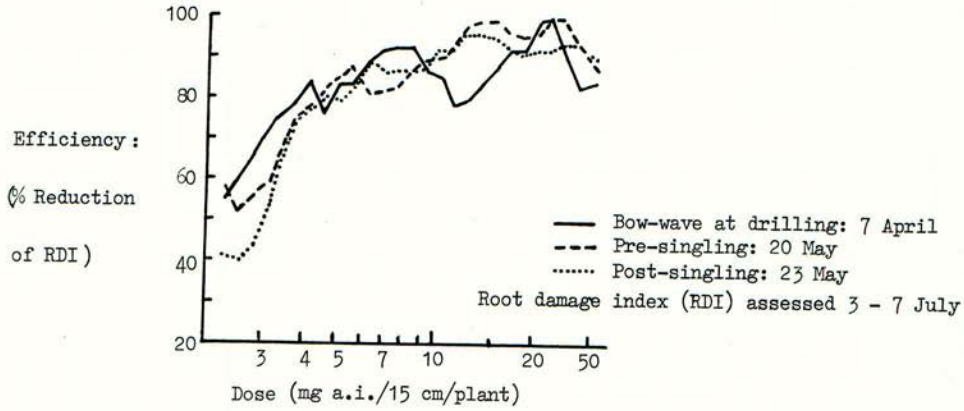
The grower is also directly responsible for the average dose of insecticide applied in accordance with a recommendation. Inaccurate calibration of machinery is well-recognised as a frequent problem, whether attributable to lack of attention or failure to compensate for the gradual drift in dose occurring as a result of wear on nozzles or moving parts of, for instance, granule applicators. The uniformity with which a dose is delivered is also an important factor not often recognised as leading to variable efficiency. The flow of granules may tend to pulse at low speeds under certain delivery conditions causing large fluctuations in the effective dose from point to point along a row and thereby reducing the average efficiency of a treatment. However, under field conditions, the slopes of dose/efficiency relationships are invariably flat compared with those normally associated with laboratory dose/mortality data. Minor variations in dose should not therefore cause major changes in efficiencies. Superficially-placed insecticide may tend to be most sensitive in this respect since it is more vulnerable to adverse influences of climate on persistence and biological activity than if deeply incorporated into the soil.

There are many ways of applying insecticides either as liquid or granular formulations to soil. Each method creates a particular distribution of concentration of active ingredients and so influences the rate of change of efficiency with changing dose, as well as the mean positions of the dose/efficiency relationships. Little is known about the precise action of insecticides against pests in soil to guide the choice of application method, and hence distribution, most suited to particular problems. There is an increasing accumulation of experimental evidence indicating that direct contact action may often not be as important as once supposed. Some of the best soil-applied insecticides have considerable fumigant potential, for instance aldrin, disulfoton, phorate or thionazin. Evidence from residue studies has shown that insecticides in soil tend to concentrate on or in the surface layers of plant root systems where they would be ingested by the pest and so act as stomach poisons. It seems likely that different combinations of these types of action may occur depending on the pest problem, the insecticide and its distribution by different application methods. Although the end-results may be similar as far as preventing pest damage is concerned, different insecticide distributions also have different effects on residues in the soil and crop, on selectivity against natural enemies of the pest and on other side-effects.

When broadcast over the soil surface, whether as liquid or granular formulations, insecticides should always be promptly incorporated, preferably as a single operation while they are being applied, to avoid substantial amounts of active ingredients being lost by volatilisation. Broadcast methods tend to be wasteful of chemical and, apart from the protection of seed-beds or close-row bed-systems of crops, they have tended to be superceded by methods which restrict the insecticide to the plant rows. For wide-spaced brassica crops, hand-treatment of individual plants with drenches or spot applications of granules is very economical and an efficient way of using insecticides to control damage by cabbage root fly larvae but it is too laborious to be practical when plant densities exceed about 20 000/ha. It is now more usual for direct-drilled or mechanically transplanted brassicas to be protected by continuous 10-15 cm wide bands of insecticides applied along the rows, even though probably a 15 cm length of band, or less, protects each plant and two-thirds or more may be wasted. Provided treatments are applied accurately, a choice of method is sometimes possible, enabling the most convenient or economic of several alternatives to be used. Direct-drilled summer cabbage was equally well protected at Wellesbourne in 1972 when chlorfenvinphos was applied either by the bow-wave method while drilling or as pre- or post-singling bands 6 and 7 weeks later respectively (Fig. 1), an instance where

Figure 1

Efficiency of cabbage root fly control on direct drilled cabbage with chlorfenvinphos applied by different methods at Wellesbourne in 1972:



choice of method was not important.

There is some circumstancial evidence that some insecticides used for cabbage root fly control on mechanically transplanted brassicas can be made more selective against the natural enemies of the pest by adopting the sub-surface method, introduced to growers in 1972, whereby the insecticide is incorporated into a zone of a few cm wide and about 10 cm deep encompassing the plant roots, rather than applying it as a band to the soil surface a few days after planting-out. Chlorfenvinphos is intrinsically relatively selective and showed no tendency in experiments at Wellesbourne to enhance cabbage root fly damage when low doses were applied to the soil surface. In contrast, plants treated with the broader-spectrum insecticides chlormephos, diazinon or fonofos were more heavily damaged than untreated plants when applied as bands to the soil surface, but not when applied by the sub-surface method.

For carrot fly control, the carrot rootlets appear to play an important part in taking-up insecticide from the soil so that the young larvae are probably killed by stomach action as they feed. Chlorfenvinphos, diazinon, dieldrin, disulfoton and phorate were all more efficient against this pest when placed 5-10 cm deep in soil, than when only 1 cm deep, and were almost equally efficient 25 cm deep where larvae do not normally penetrate. The opposite appears to apply when disulfoton or phorate are used for willow-carrot aphid control which seems to depend more on the concentration of insecticide in the soil in the immediate vicinity of the crown of the carrot than does control of carrot fly larvae. For unknown reasons, efficiency against carrot fly seems to depend more on the amount of active ingredient applied per unit area than on its concentration in any single position. There is a puzzling loss of efficiency against carrot fly larvae when insecticides are concentrated along carrot rows by the bow-wave method while drilling, compared with broadcasting and incorporating them into soil at perhaps only 1/20 of the concentration before the seed is sown (Wheatley, 1972). This is reflected in the dose differential for phorate applied by these two methods to mineral soil, a mere 15 : 25 ratio respectively. Recent research at the National Institute of Agricultural Engineering has indicated that the type of seed-drill coulter affects the granule distribution when a bow-wave method is used. This may considerably influence the efficiency of treatments since the system is likely to be sensitive to variations in distribution in the surface layer of the soil, particularly if persistence is affected.

The grower can help to minimise variations in insecticidal efficiency by selecting insecticides most suited to his particular circumstances and by ensuring that the average dose applied is that recommended and that the rate of delivery does not fluctuate unduly. Whatever application method is used, the machinery must be carefully adjusted to deliver the insecticide to the position recommended for the particular pest/crop problem. With the present range of insecticides, attention to detail is most important for attaining high efficiencies.

'Indigenous' factors in the soil environment

Given the conditions under which a crop is being grown, numerous environmental factors in the soil begin to influence efficiency after the insecticide has been applied. Most are 'indigenous' to the system and cannot be much modified by the grower, yet these post-treatment factors firmly dictate the course of subsequent events. Most are now recognised; their individual contributions to the system are known in some detail and have been the subject of several reviews (Marth, 1965; Edwards, 1966; Harris, 1972). Their inter-relationships are not, however, well-understood and it is not yet possible to relate adequately the biological activity of residues to their physical persistence in practice, taking into account the dynamic pattern of changes which occur in the course of a normal season in the field and the different conditions experienced.

The physico-chemical properties of the insecticide characterise the way in which it will behave in a given environment, particulary in determining the order of magnitude of its persistence. The indigenous environmental factors play a secondary modifying role which can be similar for chemicals of comparable properties regardless of the purpose for which they are used. Certain generalisations are therefore possible. The biological activity of an insecticide at a particular time after application depends on the distribution of concentrations of the persisting residues in the soil, their compositions and their availability. Since insecticides are usually intended to remain active in the soil for some weeks or months, only those with sufficient intrinsic stability can be used satisfactorily. Aldicarb can give an excellent control of carrot fly larvae for 10-12 weeks, even in fen soils, but by this time its active residues are diminishing so rapidly that its efficiency declines virtually to zero 8-10 weeks later. The change of phorate into its sulphoxide and its sulphone results in residues more toxic to some insects, probably including carrot fly larvae, than the parent compound. Factors such as high concentrations, (Schulz, et al., 1973), low temperatures or dry soil (Suett, private communication) which reduce the rate of change of phorate into the transient sulphoxide and more stable sulphone may temporarily reduce its toxicity and hence its efficiency.

Insecticides left on an undisturbed soil surface volatilise more readily than if they are incorporated into the soil. Even when incorporated, loss from the system by volatilisation is probably more important in accounting for diminution of residues than is generally realised, especially when the soil is subjected to frequent wetting/drying cycles as often occurs in the spring. Photochemical changes induced by sunlight may also occur in residues on the soil surface, for example, the change of trans-chlorfenvinphos into the cis-form (Suett, 1972), which may affect its toxicity.

Soil type is well-recognised as a major determinant of the physical persistence and the biological activity of pesticides. The principal factors are those associated with the organic matter content and, in mineral soils, the clay content. Degradation of residues proceeds more slowly in soils with a high organic matter content than in mineral soils and residues decline quickest in the lighter sandy mineral soils with a low clay content. Chemical and micro-biological breakdown of residues tends to proceed more rapidly when soil is moist than when it is dry.

The temperature of the soil also affects persistence and many of the present

organophosphorus insecticides cease to degrade when soil temperatures fall below about 6-7°C. Insecticides applied early in the spring will therefore remain unchanged in the soil until this critical temperature is exceeded, usually in mid-April in the U.K. When the temperature rises, degradation begins and continues approximately exponentially until the mechanism almost 'switches off' during late October/November. The residues then remain largely unchanged until the temperature rises again in the following spring, as with chlorfenvinphos (Wheatley, et al., 1972). In mid-summer, the surface layer of uncovered soil may reach temperatures of 40°C or more in the U.K., promoting loss by volatilisation and degradation. The crop canopy shading the soil surface affords some protection from extremes of temperature and also shelter from the wind, so helping to reduce loss to the atmosphere.

The physical persistence of residues, however, is not necessarily a good guide to the longevity of their biological action, the composition of the residue and the proportion freely available also being important. Climate, season and local weather determine whether the soil is wet/moist/dry or cold/warm (soil state) and induce short-term, periodic changes in the potential biological activity of residues as they become more adsorbed or desorbed on the soil particles. Some insecticides are more sensitive to changes in soil moisture status than others. Harris & Hitchon (1970) found that aldrin was 10 times more toxic to cricket nymphs in moist than in dry sandy-loam soil, methomyl was equitoxic and diazinon and disulfoton 100 times less toxic in the dry soil. Toxicity is most affected towards the very dry rather than the wet end of the moisture range in soil. Under field conditions, even a light sandy loam does not often contain much less than 5% moisture and changes in toxicity will thus be less than in the drier soil used in laboratory tests. Nevertheless, diazinon, disulfoton, phorate or dimethoate, for instance, are certainly more efficient in moist than in dry soil.

Harris (1969) classified insecticides into three groups based on the longevity of their biological action against first instar nymphs of field crickets in soil in the laboratory. He has obtained toxicity data for a wide range of compounds (Harris 1970; Harris 1973; Harris & Hitchon, 1970) but some of the groupings seem contrary to U.K. experience. For example, diazinon, chlorpyriphos (Dursban®) and disulfoton are classed as slightly residual, with dimethoate moderately residual and comparable to aldrin. U.K. experience suggests the sequence of dimethoate $\ll$ diazinon $<$ disulfoton $\ll$ chlorpyriphos $<$ aldrin. However, there is no unique way of describing biological activity quantitatively for all purposes since, in practice, it will usually be the cumulative effects on the pest population of the changes in toxicity which have occurred during the period of crop protection up to that time.

Any factors affecting the response of the pest organism to toxicants in the soil must influence the observed biological activity of a residue. The intrinsic susceptibility of the pest population is clearly important. A proportion of resistant individuals in a population sets an upper limit to the efficiency which can be attained within a normal range of dose. This is typified by the progressive deterioration in carrot fly control with dieldrin soil treatments as an increasing proportion of the population became resistant to cyclodienes during the mid-1960's (Wright & Coaker, 1968).

Temperature affects the pest's activity, and therefore the amount of toxicant it will acquire from the soil, and also the rates of physiological processes within the insect which determine its susceptibility to a given dose. The toxicity of DDT usually increases as temperature declines but most other insecticides now used have positive temperature coefficients (Harris, 1971). Their toxicity should therefore diminish as temperature falls during the autumn, and, being combined with residue decline, should progressively reduce efficiency. However, this may or may not be offset by greater availability of the toxicant as the soil becomes more moist. In experiments to evaluate insecticides against carrot fly at Wellesbourne and elsewhere, the efficiency of some did, in fact, increase during the autumn but more usually it has declined. The uptake of residues by the maturing crop also diminishes

at this time of the year (Suett, 1971), so adding to a series of events which may account for at least some of the difficulties in controlling carrot fly damage during late autumn and winter, especially in black fen soils.

In view of the many factors involved, and their complex inter-relationships it seems remarkable that insecticides can be consistently effective in soil. Yet for many years few problems arose with the use of aldrin or dieldrin and experiments suggest that violent fluctuations do not often occur in the efficiencies of the newer chemicals provided they are carefully and correctly applied.

FACTORS INFLUENCING EFFECTIVENESS

Effectiveness is concerned with the success or failure of an insecticidal or other treatment to provide an assured, economically satisfactory yield of crop suitable for a particular market outlet. It embraces the popular meaning of 'control', or crop protection and, in contrast to efficiency, it is difficult to assess in absolute terms by experiments or to define in any single way. Yields from commercially treated crops are undoubtedly the best criteria of effectiveness, but the levels of effectiveness required differ for each pest/crop/market problem (Wheatley & Coaker, 1970; Wheatley, 1971a). Attempts to relate the effectiveness of treatments to factors such as the chemical, its dose, the application method, the soil type or even the treatment's efficiency, are not usually very successful because of the many other influences in the system between applying the chemical and harvesting the crop.

Table 2

Relationships of factors influencing effectiveness

Factors	Effects		
Potential efficiency + natural control + infestation	Net efficiency	Residual infestation	Crop loss
Crop + environment + insecticide	Crop growth →	Crop potential	
Crop potential − crop loss	Marketable yield →		EFFECTIVENESS

The efficiency of a treatment and its effectiveness are linked by the factors naturally controlling the pest and the intensity of its attack, by those determining crop growth, and by environmental influences (Table 2). Since their inter-relationships are very important and many of these factors operate simultaneously, they cannot be studied sensibly in isolation.

Net efficiency

Efficiency as just discussed, is more strictly the potential efficiency of a treatment, because the effects of other agents, both physical and biological, are complementary to those of the insecticide. Physical factors associated with weather and climate induce proportional changes in the densities of pest populations and the these combine multiplicatively with insecticidal efficiency since they act together independently of one another. Physical factors will not now be further considered in this context. The efficiencies of biological controlling agents, such as parasites and predators, however, are dependent on the density of the pest. Furthermore, they may themselves be affected by the insecticide so that their contributions to control of the pest may be very much affected by the treatment as a whole (chemical and application method). These effects are usually confounded in practice and so it is their net efficiency which is normally assessed in field tests. Because of the

additional processes involved, this will be more variable than the potential efficiency of the insecticide treatment.

The consequences of interfering with the beneficial activities of predatory beetles which naturally control cabbage root fly populations in the soil are well-known (Wright, et al., 1960; Mowat & Coaker, 1967; Coaker & Finch, 1971). While aldrin and dieldrin were still very efficient against this pest, the practical importance of the predators was not very evident, but present insecticides are less efficient and commonly reduce its damage on brassicas by only 70-90%. Even though the potential efficiencies of selective and relatively non-selective insecticides may be similar, non-selective insecticides are liable to act against either the pest, the predator or both depending on the dose. Their net efficiencies therefore will usually be less than for their selective counterparts which retain the contribution of the natural enemies. The results of recent log-dose trials at Wellesbourne support this. At low doses, broad spectrum insecticides have often enhanced damage by cabbage root fly larvae whereas this has never occurred with chlorfenvinphos which was shown by Mowatt & Coaker(1967) to be relatively selective.

Relationships between net efficiency, the residual infestation and its multiplication rate

The numbers of insects surviving to damage the crop, the residual infestation after treatment, is essentially a product of net efficiency and the magnitude of the attacking pest population. The behaviour of the survivors will then be influenced by other factors.

Only a limited number of insects can be allowed to survive per unit of crop if the loss is not to exceed the economic injury level. Hence, the larger the infestation, the greater the efficiency needed to counter it. Treatments with low efficiencies will not cope adequately with even moderate attacks and, as a guide, those with efficiencies less than 50% should not be considered as practical control measures against any pest. Efficiencies of 50-70% may be adequate for some purposes if infestations are low but if at all possible, they should be improved or superceded. For problems involving indirect damage (see Wheatley & Coaker, 1970; Wheatley 1974), efficiencies in excess of 70% will usually be adequate to control, for example, cabbage root fly damage on transplanted brassicas, except when severe infestations occur, provided the protection is maintained for at least 4 weeks after planting. Problems involving premature plant death or direct damage to the marketed part of the crop usually require that efficiencies in excess of 90% must be maintained until harvest, for example for carrot fly control. It is questionable whether treatments which do not achieve these minimum efficiencies should be approved for use, since their inability to protect crops satisfactorily from even moderate infestations can mislead users and, if they are relatively non-selective, there is a real risk that they may enhance pest populations and hence crop damage. The importance of the pest population surviving treatment can be considerably modified by factors such as climate (temperature and rainfall) and suitability of the host-plant (susceptibility/resistance to attack) which affect the growth and multiplication rates of the survivors and hence their potential to damage the crop.

Relationships between crop growth, environment and the insecticide

This very complex part of the system sets a potential for crop yield in any particular circumstance of soil, climate, cultivar and agronomic practices. A full discussion is beyond the scope of this paper. However, some insecticides seem to induce growth responses in crops (Martin, 1972) quite apart from relieving the plant from pest stresses or being acutely phytotoxic. In this way, insecticides may modify the crop's potential.

Several types of effect on cabbage, cauliflowers, Brussels sprouts and carrots have been observed in log-dose trials at Wellesbourne involving candidate and approved insecticides. In 1971, the diameters of summer cauliflower curds failed to increase with increasing dose of chlorfenvinphos but increased progressively when diazinon was used (Wheatley, 1971b) despite less reduction of root damage by cabbage root fly larvae. A similar result occurred on summer cabbage in 1972 when there was no significant increase in yield as a result of using chlorfenvinphos although it efficiently controlled a cabbage root fly infestation causing 40 to 65% root damage on untreated plants (Figure 1). The yield of cauliflowers has also been observed to be less improved by the use of chlorfenvinphos than by dimethoate treatments, although the former protects the plant roots longer from damage. Even applying chlorfenvinphos in different ways apparently altered the proportions of small, medium or large Brussels sprouts in a log-dose experiment at Wellesbourne in 1972. Either chlorfenvinphos slightly inhibits the growth of brassicas or certain other insecticides stimulate their growth. Different effects of insecticides on the survival of carrot seedlings have also been observed in experiments (Wheatley, 1974), an undesirable complication for the grower who calculates seed-rates to achieve the desired size-range of carrots for a specific market outlet. Such effects have considerable economic implications since they may add to or detract from the overall effectiveness of treatments.

Relationship between pest infestation and the crop

Pest damage assessments are concerned with the relationships between the intensity of pest attack and crop loss, the suppression of crop yield below its potential. The economic principles have been discussed by Strickland (1970). Only certain general aspects will be considered here.

Crop pests may affect seedling survival, the growth of the plant, or directly damage the marketed produce (Wheatley & Coaker, 1970; Wheatley, 1974) and some cause all three types of damage. Seedlings of most crops need to be protected to achieve an optimum plant density and thereby to maximise yield. For precision-spaced, direct-drilled crops, treatments must usually ensure that at least 95% of the seedlings survive, leaving only small residual pest populations, often difficult to detect in soil. Good protection is needed even for continuously drilled crops such as carrots since reduction in plant density caused by pests may affect the size-range and thus the suitability of the produce for a particular market. Compensatory growth usually reduces uniformity and is not desirable in most vegetable crops. Pest damage to the non-marketed part of a crop tends to suppress the plant growth. The benefit to be derived from a successful treatment will depend on the conditions for growth and the responses to agronomic treatments may, in turn, depend on pest damage. Thus the effects of moderate injury to roots by the cabbage root fly larvae can be partly offset by irrigation or opportune rainfall, although cabbages and cauliflowers respond best to irrigation when they are protected from this pest throughout their growth (Coaker, 1965). The relationships between efficiency and effectiveness of treatments seem to be most easily understood in the case of direct damage problems, such as carrot fly mining in carrots or parsnips, or cabbage root fly larvae in Brussels sprout buttons. The objectives for carrot fly control for particular markets can be defined mathematically, indicating how firm criteria for treatment efficiencies are possible (Wheatley, 1969).

Plant density is an important factor to relate to the residual infestation, since together they determine the average number of insects per plant. High plant densities usually result in fewer plants being damaged, or in less damage being caused to each, by a given residual insect population. However, a dense crop may also attract larger numbers of the pest if 'surplus' insects are available nearby and in carrot fly attacks, this can partly compensate for the dilution effect of a high plant density. At Wellesbourne, an eight-fold increase in density of parsnips resulted in an estimated two- to three-fold increase in the carrot fly larval

population, but the numbers of parsnips damaged declined from 77 to 60% (Wheatley & Hardman, 1958). Similar effects of plant density have also been observed with carrots (Hardman & Wheatley, 1971).

DISCUSSION

There appears to be two main areas to which effort needs to be directed to improve effectiveness of soil-applied insecticides. Firstly, a more thorough understanding of the insecticide/soil relationships are needed so that more efficient treatments can be devised. Technical, economic and legislative restraints may make it difficult to introduce more potent, stable or selective insecticides, but it may be possible to improve formulations and application methods. Research is needed to determine the optimum distributions for insecticides in soil for each problem so that the engineer can improve the design of application machinery. Meanwhile, present knowledge of the behaviour of insecticides in soil needs to be more widely disseminated and more fully utilised. Manipulation of the soil environment to increase the biological activity of the persisting residues may occasionally be possible, as by irrigation, but this is not likely to be feasible in many practical circumstances. Secondly, much greater attention will have to be given to the numerous biological factors interposed between efficiency and effectiveness in the system. Positive efforts are needed to reduce or limit infestations by avoiding agronomic practices liable to enhance pest attack, such as seasonal overlap of crops, growing alternative host-crops in close proximity to each other, ultra-intensive cropping and the use of hypersusceptible cultivars. Farm hygiene also needs to be improved and good crop growth maintained. All pesticides should be checked for possible chronic adverse effects on plants.

The analysis of pest control systems helps to identify key factors and hence areas for research and to indicate the type of data needed from evaluation trials so that candidate chemicals can be tested more economically than at present. Future pest control procedures will probably involve the integration of different methods, including the use of insecticides, sterile male release, attractants and lethal lures resistant cultivars, genetic manipulation and revision of cropping systems. It is difficult to conceive how optimum strategies can be devised without a systems approach in some degree. Even the outline structure described here illustrates how the results from disjointed studies on new chemicals, formulations, application methods, insecticide residues, behaviour and biological activity in soil, forecasting of infestations, pest damage assessments, biological control, agroecology and effects of climate and weather could contribute to a better understanding of pest control procedures. We may eventually be able to devise more effective treatments logically rather than relying on ad hoc approaches as in the past.

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CONTROL OF SOIL-BORNE PATHOGENS OF CROPS OTHER THAN CEREALS

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Summary Soil-borne diseases play a vital part in limiting the productivity of many crops and are particularly important in some of the high value cash crops, especially those which are grown in mono-crop systems. There have been important advances made in recent years in the control of these diseases. This has been most evident in the field of chemical control, but recent research has made other methods of control available. These include biological control, the use of resistant or tolerant cultivars, and the placement of physical barriers between the crop and the affected soil. These recent innovations will be reviewed and their value to the agricultural industry assessed.

Résumé Les maladies portées par le sol jouent un rôle important en limitant la production de plusieurs récoltes, particulièrement les récoltes de grande valeur monetaire et speciallement les récoltes produites par systèmes mono. Recemment il y a eu des progres importants au sujet du control des maladies du sol. Cela est très évident dans le control chimique, mais grâce à des recentes recherches, d'autres methodes de control sont disponibles. Celles-ci sont le control biologique, l'usage des cultivars qui sont resistants ou tolerants et le placement des barrières physiques entre les récoltes et le sol affecté. Ces recentes innovations seront revues et leurs valeurs par rapport à l'industrie agricole sera evaluée.

INTRODUCTION

Historically the most important method of controlling soil-borne pathogens has been by crop rotation, but in modern farming and particularly in horticulture, intensive cropping systems and indeed mono-cropping systems are now widely adopted. The pressures which have brought about this change have been mainly economic, but it is also true that the development of techniques for controlling soil-borne diseases has played an important role in the wider adoption of highly intensive cropping systems.

These techniques have generally been developed and most widely used for glasshouse crops, but a greater awareness of the losses associated with soil-borne disease has led to the development of sophisticated control measures for use in vegetables, fruit and other field grown crops.

When deciding which method of disease control to adopt for a particular crop the grower has several interacting factors to consider. The occurrence of a

specific disease problem in the previous crop may make treatment essential, but even where disease levels appear to be low or non-existent, it is difficult to be sure that some build-up of disease during the following crop will not affect yields, and for this reason insurance treatments are applied. The type of treatments to apply will depend on the numbers and type of soil-borne pathogens which affect the crop and which of these are known or suspected to be present in the soil.

The expected monetary return from treatment must also be assessed and this is especially true when a choice of several treatments is available.

The many different methods available to control soil-borne diseases can be classified into chemical treatments, physical barriers between crop and contaminated soil, tolerant or resistant cultivars, biological control and soil pasteurisation.

Some of these methods are "blunderbuss" treatments which aim to control a broad spectrum of soil-borne pathogens, whilst some are very specific. Some have been effectively used for many years, others are comparatively new and their wide adoption awaits the further testing of their efficiency and a wider assessment of their advantages and possible disadvantages. It is impossible in the time and space available to consider all of the methods that are currently available for the control of soil-borne pathogens and this review is restricted to the examination of certain recent developments.

Chemical Control

There are two aspects of chemical control which must be considered as having made a significant advance in soil-borne disease control in recent years. Firstly the introduction of chemicals which can be applied to the growing crop or to planting material, and secondly the introduction of new and expanding methods of soil chemical fumigation.

Of the chemicals which can be applied directly to the plant or to the soil in which the plant is grown, the benzimidazole and related chemicals have had the greatest impact. These chemicals have been shown to be effective against many soil-borne diseases which in the past had proved difficult or impossible to control.

Benomyl applied as pre-planting dips or post-planting drenches to transplanted strawberry runners has given effective control of *Verticillium dahliae* (Lockhart *et al.*, 1969; Jordan, 1972a and b; Talboys and Frick, 1971) and this technique is now being widely adopted by strawberry growers and has allowed the production of economically viable crops of susceptible early varieties to be grown on land where such crops had previously failed. Post-planting drenches have also reduced the expression of wilt symptoms in strawberries grown for runner production. However, benomyl is fungistatic in action and infected strawberry runners so treated are potentially wilt carriers. Such treatments, however, have given marked increases in runner production even in the absence of wilt (Gwynne, 1973) and could provide a valuable aid to increased productivity of strawberry runner beds.

Post-planting benomyl drenches have also proved effective in controlling wilt (*Verticillium cinerescens*) of carnation. A drench applied as soon as wilt appeared in the crop and again 14 days later reduced levels of infection from 50% to 12% over a 10-month cropping period, but similar treatments failed to give control of carnation wilt caused by *Fusarium oxysporum* f. sp. *dianthi* (Evans, 1973a).

Other soil-borne diseases which have been effectively controlled by benzimidazole or thiophanate fungicides applied as drenches to the soil or as seed or transplant treatments include stem rot (Didymella lycopersici) of tomato (Channon, 1972), club root (Plasmodiophora brassicae) of brassicae (Jacobsen and Williams, 1970; Buczacki, 1973) and white rot (Sclerotium cepivorum) of onion (Ryan et al., 1971).

These treatments clearly have marked advantages over previous control measures, particularly where they replace mercury treatments for the control of such diseases as club root of brassicae and white rot of onion. However, benzimidazole and thiophanate fungicides have been shown to seriously deplete earthworm populations in soil (Cook and Burchill, 1972; Stringer et al., 1972) and the deleterious effects on some crops of the absence of surface-feeding earthworms may prove to override any advantages shown by these treatments. It has also been shown that fungal strains tolerant to benzimidazole and thiophanate chemicals develop quite rapidly after repeated treatments with these fungicides. This is especially so with grey mould (Botrytis cinerea) and powdery mildew (Erysiphe cichoracearum) of cucumber. The development of tolerant isolates of soil-borne pathogens must therefore be carefully monitored, especially where such chemicals are being used for their control, and research and development work should continue to explore possible alternatives to benzimidazole and thiophanate fungicides for the control of soil-borne diseases.

Chemicals have also recently been tested for the control of some soil-borne diseases which are not controlled by the benzimidazole and thiophanate systemic fungicides.

Terrazole and captafol have been shown to be effective in the control of root and stem rot (Phytophthora cinnamomi) of conifers. In trials in which plants of Chamaecyparis lawsoniana Ellwoodii grown in pots containing contaminated soil were drenched at fortnightly intervals, both treatments reduced disease levels from 80% to nil. There were, however, slight phytotoxic effects from captafol (Evans, 1973b).

The same fungicides have been used in recent trials to control red core (Phytophthora fragariae) of strawberry (Upstone, 1973). In one trial soil drenches of captafol or terrazole at autumn planting and in the following spring reduced red core levels from 7.3% to 0.3% and 0.7% respectively.

Further development work is required to determine optimum levels and times of fungicide applications, however, before such treatments can be recommended for commercial use.

The second widely used method of chemical control of soil-borne diseases is chemical fumigation before planting. Chemical fumigants have been used for many years in the glasshouse industry and have included such chemicals as metham sodium, dazomet, chloropicrin and DD. These chemicals are effective against a wide range of soil pathogens but their main disadvantage is the long interval between treatment and planting necessary for the clearance of toxic residues. Soil fumigation with methyl bromide has become widely adopted for the control of soil-borne diseases of glasshouse crops because it lacks this disadvantage. It is applied to soil beds well sealed with polythene sheeting, either by the canister method in which liquid methyl bromide is allowed to evaporate from cans standing beneath the sheeting, or by the heated coil method where the liquid is vaporised by passage through a warmed metal coil and led beneath the sheeting through plastic tubing. The latter method is regarded as more efficient (Galley and Hague, 1967) and is being used on an increasing scale. Most crops can be planted seven days after treatment, but concentrations of inorganic bromide may build up

which can cause damage to certain crops, such as carnations (Williamson, 1953). However, further work is required to determine optimum concentrations and times of treatment for specific pathogens.

Methyl bromide is finding increasing use either alone or in combination with chloropicrin in the control of soil-borne pathogens of field crops. Mixtures of methyl bromide and chloropicrin have given good control of root diseases of strawberries, particularly of Rhizoctonia fragariae in California (Wilhelm et al., 1972). This pathogen has recently been isolated from the roots of strawberries in crops in Kent, and appears to be partly or solely responsible for the poor growth and cropping of some strawberry cultivars when they are replanted on to land which has been frequently cropped with strawberries (Wiggell and Griffin, 1973). Methyl bromide and chloropicrin are currently being tested as soil fumigants for the control of this disease in the UK.

Chloropicrin is also being widely used for the control of specific apple replant disease and of cherry replant disease in the UK, but it is a noxious material to handle and apply, and supplies of the chemical are becoming very limited. Recent work at East Malling Research Station has indicated that the cherry replant disease can be controlled by soil drenches of benomyl which are effective against Thielaviopsis basicola, the causal organism of the disease (Hoestra, 1965). A replacement for chloropicrin for the control of specific apple replant disease, the cause of which is unknown, is urgently required.

Physical Barriers

Physical barriers isolating the crop from affected soil have been used as a means of controlling soil-borne plant diseases for several years. The growing of carnations in isolated or raised concrete beds is a technique which is well established and widely used for the control of wilt diseases caused by Verticillium cinerescens and Fusarium oxysporum f. sp. dianthi. Straw bales or wads have been used to isolate tomatoes and cucumbers from contaminated soils (Anon, 1965) and polythene barriers separating contaminated soil from beds made from sterilised soil or compost have been used for chrysanthemums and other crops.

A promising new technique where tomatoes and cucumbers are grown with their roots contained within polythene tubing through which a nutrient solution is circulated is currently being investigated at the Glasshouse Crops Research Institute (Cooper, 1973). This would appear to be a technique of considerable promise in controlling soil-borne diseases of a number of crops and it is being tested on a limited scale on commercial nurseries. However, commercial growers applying this system to carnation growing have already sought recommendations for fungicides which can be incorporated with the nutrient solution to reduce levels of wilt diseases which may have been introduced into the system by planting wilt-infected carnation cuttings.

Tolerant or Resistant Cultivars

Biological barriers to infection with soil-borne pathogens, whereby grafts of susceptible cultivars are topworked onto resistant rootstocks, have been widely used in the glasshouse industry. Rootstocks resistant to wilt (Fusarium oxysporum) of cucumbers and to wilt (Verticillium dahliae and Fusarium oxysporum) and stem rot (Didymella lycopersici) of tomato are effectively used in the control of these diseases.

Similar techniques are used to reduce the incidence of collar rot (Phytophthora cactorum and P. syringae) of apples where highworked resistant

rootstocks are used to prevent infection of susceptible scion cultivars when apples are replanted on to contaminated soil (Sewell and Wilson, 1964).

The breeding of tolerant or resistant cultivars for the control of some soil-borne diseases has, however, had mixed results. Considerable success has been achieved in breeding cultivars of hop tolerant to the severe (progressive) strains of wilt (Verticillium albo-atrum). Indeed the tolerance to wilt of the high alpha-acid yielding cultivar Wye Target (Neve, 1972) could be the basis of a future viable hop industry in areas such as the Weald of Kent, where the disease is endemic.

There are several other instances of successful breeding of resistance to soil-borne pathogens. However, there has only been partial success to breeding tolerance or resistance to red core (Phytophthora fragariae) of strawberry. Cultivars have been released which have shown resistance to some of the 11 races of the fungus but no cultivar has shown resistance to all races and a search for field tolerance in commercially acceptable cultivars appears to be a continuing process. There is a definite requirement for an examination to be made of the tolerance or resistance of hardy nursery stock cultivars to stem rot (Phytophthora cinnamomi) so that guidance can be given on crop species which can be planted on to contaminated sites, and a study of the existence of physiological races of this and several other soil-borne pathogens could usefully be made so that plant breeders and plant pathologists can be made more fully aware of the problems to be overcome in successfully breeding cultivars tolerant to soil-borne pathogens.

Biological Control

Biological control of soil-borne pathogens is a technique to which researchers have only recently applied themselves and some encouraging results are already available. That some micro-organisms are antagonistic to particular soil-borne pathogens has been used in the past in integrated control systems, and has been the basis by which some chemical soil fumigant treatments have been successful. For example, carbon bisulphide and methyl bromide fumigation to control Armillaria mellea in orchard and forest soils has been used to modify the soil microflora and so increase populations of Trichoderma viride known to be antagonistic to the pathogen (Bliss, 1951; Ohr and Munnecke, 1973). Work in Australia has also shown that antagonism of soil microflora to Phytophthora cinnamomi can be increased in some soils, especially when the soil pH is modified by heavy liming (Broadbent and Baker, 1973). Soil populations of Aspergillus ustus, A. flavus and Penicillium spp. have recently been demonstrated to play a significant role in the destruction of sclerotia of Solerotinia sclerotiorum, a soil-borne pathogen of many crop species (Rai and Saxena, 1973), and Bacillus isolates from soil have been shown to be antagonistic to Rhizoctonia solani and when added to contaminated soil have reduced the incidence of "damping-off" caused by the pathogen (Broadbent et al., 1971).

Soil amendment by chemicals or by other treatments to increase antagonists is therefore a means by which some soil pathogens can be controlled, but the possibility should not be overlooked that in so doing, other pathogens not specifically antagonised may increase and bring about even heavier crop losses. Perhaps the most commercially developed method of biological control is exhibited in the protection of tomatoes from wild strains of tobacco mosaic virus by inoculation with the mild strain M II-16 (Rast, 1972). This strain is now widely used in the tomato industry after extensive tests had shown a mean 6.8% in yield of inoculated over uninoculated crops in 26 trials monitored by plant pathologists of the Agricultural Development and Advisory Service (Upstone, 1972).

Success has also been reported in the cross protection of cherry from the soil-borne raspberry ringspot virus by using stabilised and mild strains of the virus (Dunez and Fleury, 1973) and it is possible that a wider use of similar techniques will be made for controlling soil-borne virus diseases in the future.

Soil Pasteurisation

Of the methods used for controlling soil-borne diseases, perhaps the most effective is soil pasteurisation by heat; but the technique is virtually limited to glasshouse soils and is invariably carried out using steam. There are many different methods by which steam is introduced into glasshouse soils but most of them are variations on two basic concepts. In one the steam is introduced below the surface of the soil and passes upwards through it and in the other steam is applied under pressure at the surface and passes downwards. The most widely used techniques in modern glasshouse husbandry include "sheet steaming" where steam is introduced beneath PVC or butyl rubber sheets or "coffin" steaming where a metal box replaces the PVC sheeting. However, the use of mobile steaming grids has recently been revived following the development of a model by the National Institute of Agricultural Engineering which will provide steam penetration to a depth of 14 or 15 inches. This method is gaining favour and has proved more effective for the control of some soil-borne diseases of carnations and other crops than other steaming methods.

DISCUSSION

Recent developments in the control of soil-borne pathogens have provided new methods of disease control which are quickly gaining favour in the agricultural and horticultural industry. Many of them are useful tools when applied singly, but there is an increasing awareness that several different techniques will need to be used at any one time for the control of the many soil-borne pathogens which can attack even a single crop species. The further integration of these different methods will almost certainly be the area in which future developments will proceed, and it is likely that integrated control will have to become more widely developed to reduce levels of disease in cropping systems which will almost certainly become more intensive.

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THE OCCURRENCE OF A SECOND RACE OF THE TOMATO FUSARIUM WILT IN THE GLASSHOUSE

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Summary An isolate of the tomato wilt pathogen Fusarium oxysporum f.sp. lycopersici, different from the common strain (race 0) and virulent towards cultivars carrying the race 0-resistance gene I, was isolated from one of five diseased plants found growing in a glasshouse crop of resistant cultivars at a nursery in Lea Valley, Essex, during 1970. This is the first known report of a specialised pathogenic strain of this organism occurring both in Britain and in a commercial glasshouse crop. The possible implications of this event are discussed in relation to similar experiences with outdoor crops and the necessity for integrated control measures outlined. A modified system for numbering races of the pathogen is proposed together with reasons for its considered adoption.

Résumé Un isole du microbe pathogène de la flétrissure de la tomate, Fusarium oxysporum f.sp. lycopersici, qui diffère de la souche commune (race 0) et qui est virulent pour les cultivars portant la race 0- gène résistance I, a été isolé d'une des cinq plantes atteintes qui se trouvaient dans une culture en serre de cultivars résistants dans une pepinière de la Lea Valley, Essex, au cours de 1970. C'est la première note qu'on ait d'une souche spécialisée pathogénique de cet organisme se produisant en même temps en Grande-Bretagne et dans une culture de commerce en serre. On discute les possibilités impliquées dans cet évènement par rapport à des expériences similaires avec des cultures en plein air et l'on esquisse la nécessité de mesures intégrantes de contrôle. On propose une méthode modifiée de numéroter les races de microbes pathogènes, aussi des raisons d'en considérer l'adoption.

INTRODUCTION

The use of monogenic resistant tomato cultivars to combat vascular wilt disease caused by the fungus Fusarium oxysporum f.sp. lycopersici originated in the U.S.A.

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in the early 1940's with the release of the cultivar 'Pan America' (Porte & Walker, 1941). This incorporated the major resistance gene I-1, which was discovered in an accession of the wild species Lycopersicon pimpinellifolium (Bohn & Tucker, 1940). It has since been used extensively by plant breeders in all countries where Fusarium wilt has been a problem, and commercially the impact and success of monogenic resistance can immediately be gauged from the length of time that resistant cultivars have been grown. Furthermore, in the U.S.A., the use of resistant cultivars did not seem to herald the rapid appearance of new races able to overcome resistance, and it has been cited by numerous authors, e.g., Van der Plank (1968) as one of the few examples of 'stable' monogenic resistance in commercial use. Thus, although Alexander & Tucker (1945) first reported a second race of Fusarium as existing in the early 1940's, it was twenty years later in 1961 before Stall (1961) in Florida, U.S.A., published the first account of its attacking commercial crops of resistant cultivars. This second race of Fusarium is now known in other parts of the U.S.A., in Brazil and nearer home in Mediterranean countries such as Israel and Morocco (Walker, 1971) where experiences have largely been the same as in Florida.

On the question of race nomenclature, traditionally the two known strains of Fusarium have been referred to as races 1 and 2, based on the historical precedent set by Gerdemann and Finley (1952). Unfortunately this nomenclature was introduced before the logical system of Flor which takes in to account the now generally accepted gene-for-gene relationship between host and pathogen (Flor, 1955; Van der Plank, 1968). We propose that in order to bring the system of nomenclature up to date, by equating the virulence genes in the pathogen with the genes for resistance in the host, that the two strains be referred to as races 0 and 1. This conforms with the gene-for-gene concept of Flor (1955) and follows the now internationally agreed system of nomenclature for races of Phytophthora infestans proposed by Black, Mastenbroeck, Mills & Peterson (1953) and since adopted for races of tomato leaf mould (Cladosporium fulvum), (Day, 1956), tomato strains of T.M.V. (Pelham, 1969) and many others quoted by Person (1968). A fuller, more detailed account of the proposed scheme is not appropriate to this paper and is to be published elsewhere.

DISEASE OUTBREAK

In May, 1970, a situation arose in the Lea Valley, Essex, which led to the discovery of the specialised strain (race 1) of Fusarium infecting a resistant cultivar for the first time in Britain and, to the authors' knowledge, for the first time in a commercial glasshouse crop.

Following an outbreak of Fusarium wilt at a nursery growing the susceptible tomato cultivars 'Potentate' and 'Kingley Cross', the attack became so severe in one house that the entire crop had to be removed. In order to assess available material for use in next year's crop, the grower decided to replant in the same infected soil but using the Glasshouse Crops Research Institute bred Fusarium-resistant cultivars, 'Cudlow Cross', 'Fontwell Cross', 'Grenadier' (J396), J460 and J461. During the subsequent growth of a crop comprising around 1,000 plants, five plants were found with symptoms of Fusarium wilt. Under normal circumstances these would probably have been ignored and not considered any real great cause for alarm. However, on this occasion it was decided to examine more closely the pathogenic virulence of isolates obtained from the five infected plants, and two of these, selected on the basis of their aggressiveness, were sent to G.C.R.I. to be race typed using differential hosts.

METHODS

Seedling pathogenicity tests were performed using inoculum consisting of standardised conidial suspensions (10^7 spores/ml) prepared from the two Lea Valley isolates (B and D), together with an isolate of the common strain (race 0) from

Guernsey and an isolate of the specialised strain (race 1) obtained from Florida, U.S.A. The differential hosts consisted of susceptible British pure-breeding cultivars together with their race O-resistant near isogenic counterparts, while resistance to race 1 was obtained using the U.S.A. cultivar 'Walter', which carries the gene I-2 for resistance to race 1 in addition to the race O resistance gene I-1.

RESULTS

A series of test inoculations, carried out at different times under standardised conditions of temperature and soil conditions but using different paired susceptible/resistant cultivars, confirmed that the two Lea Valley isolates differed in respect of their pathogenic virulence towards the various hosts. Isolate B gave rise to a host reaction similar to that of the Guernsey isolate and typical of race O, whereas the virulence of isolate D matched that of the race 1 isolate from U.S.A., producing disease symptoms on all susceptible cultivars and their race O-resistant counterparts. The cultivar 'Walter' proved to be resistant to all isolates at all times. A summary of the results of all screening tests is presented in Table 1.

Table 1

Virulence of four isolates of Fusarium oxysporum f.sp. lycopersici on tomato differential hosts

CULTIVAR	GENOTYPE	ISOLATES: LEA VALLEY B		LEA VALLEY D		GUERNSEY race O		U.S.A. race 1	
		T	%	T	%	T	%	T	%
susceptible	+/+; +/+	20	90	116	90	90	96	40	100
race O-resistant	I-1/I-1; +/+	20	0	96	78	98	6	40	100
race 1-resistant (Walter)	I-1/I-1; I-2/I-2			96	1	79	0	40	0

T = number of plants tested % = percentage infected

DISCUSSION

The continuing success or early failure of disease resistance when exploited in commercial practice depends to a large extent on the degree to which the pathogen is allowed to multiply and spread. The greater the number of spores the pathogen is allowed to produce, the greater the chances of new pathogenic strains arising, as a result of genetic recombination or mutation. If allowed to establish, such strains could nullify the efforts of the plant breeder. The soilborne nature of the tomato wilt pathogens restricts spore mobility, and the multiplication rate under such conditions is generally low (Garrett, 1970). If, however, the grower allows his standards of crop hygiene to fall and if infected plants are not removed, the fungus can sporulate on the outer tissues of dying plants. This introduces a new problem

for once the spores are allowed to become airborne, spore mobility is increased and the chances of disease spread become very much greater. In this context it is important to remember that resistance to Fusarium conferred by the gene I-1 is really a tolerance to infection and cannot strictly be regarded as the 'immunity' which the gene symbol denotes. Normally, however, if infection of resistant plants does take place, the fungus is contained within the vascular system of the root or basal region of the stem. In such an instance the only indications that infection has taken place are the accompanying thin streaks of vascular discolouration, outwardly the plant will appear completely healthy. Although diseased resistant plants do occur in the field, their presence is rarely in large enough numbers to be any real cause for alarm, and as a consequence routine checks are seldom made to ascertain whether the infecting strain is race 0 or 1.

It is interesting that the strain of F. oxysporum f.sp. lycopersici, which Alexander and Tucker isolated in 1941 from the stem of a plant of the susceptible cultivar 'Livingston's Globe', proved to be race 1. This meant that the specialised strain had appeared before the general release of resistant cultivars such as 'Pan America' and its occurrence was apparently not associated with, or influenced by, the gene I-1. The extent of the outbreak was never determined. Repeated attempts to re-isolate it in later years failed. Cirulli & Alexander (1966) considered this to be attributable to the annual steam sterilization which the glasshouse soil received. However, it could also be argued that race 1 would have disappeared in any event, since it would have been selective disadvantage in the presence of race 0 growing on susceptible plants. The mutation from race 0 to race 1 was most likely a chance event which has occurred on numerous occasions both before and since it was first identified.

Stall's (1961) discovery of race 1 concentrated in a five acre site at Delray Beach, Florida, U.S.A., was the first experience of race 1 on a commercial scale, and was disturbing since all the plants carried the 'Pan America' resistance factor I-1. This provided the selective screen for the new race and there were fears that it would spread in to surrounding tomato growing areas. It was not until 1964, however, that a further outbreak of race 1 was encountered and this occurred in the Manattee county area of Florida some 150 miles from the original infection site at Delray Beach (Jones & Littrell, 1965). In 1966 a survey of Fusarium wilt in Florida revealed that the presence of race 1 was limited to these two areas. It was presumed that the two isolates were of the same origin and that the spread had resulted from the frequent movement of vehicles between the two areas (Jones, 1966). It was not until the late 1960's that race 1 was shown to have become a statewide problem, by which time, a new cultivar 'Walter' had been released with resistance to races 0 and 1 by incorporation of a second resistance gene I-2 (Strobel, Burgis, Everett & Hayslip, 1969); by 1971 this had become the most widely grown cultivar in Florida (Crill, Jones, Burgis & Woltz, 1972).

The large number of race 1 infected plants discovered by Stall (1961) in the original Florida disease outbreak was spread over 400 acres by the time that the disease was discovered although concentrated in just five of those acres. This represented a secondary stage in the development of the disease epidemic, and this probably resulted from the movement of airborne spores. It was obviously not possible to even estimate the actual number of primary race 1 infection sites or when and where these occurred. This is important as the situation contrasts markedly with that of glasshouse crops, where, because of the greater attention generally paid to individual plants, disease detection is easier. The five diseased plants detected in the case of the Lea Valley outbreak were distributed at random in the glasshouse and probably represented primary infection sites. In hind sight it is a great pity therefore that only two of the five isolates were race typed.

Since the 1970 Essex experience, isolates of Fusarium - taken from infected race 0 - resistant plants were requested through the Agricultural Development and

Advisory Service and Guernsey Horticultural Advisory Service and several of these were race typed at G.C.R.I. but none proved to be race 1.

There have been no previous reports of Fusarium race 1 in Britain; Hubbeling (1973) reported a similar occurrence of race 1 in 1971 in the Westland area of Holland but this also appeared to be an isolated example and nothing more has been reported. This is probably due to the annual sterilization of the glasshouse border soil undertaken between successive crops. The absence of race 1 from the Lea Valley in 1971, and since, supports this explanation and strengthens the case for using integrated systems of disease control for glasshouse crops - especially where mono-cropping is practised.

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INDIRECT CONTROL OF SOIL FUNGI

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Summary Certain concentrations of sodium and 1-S-n-octylisothiouronium indoleacetates, indolepropionates, indolebutyrates, phenoxy-, p-chlorophenoxy-, p-bromophenoxy-, and 2:4-dichlorophenoxyacetates were able to protect cotton, flax and tomato seedlings against soil fungi in the soil. This might be due to slight physiological disturbances of the plant tissues. In the laboratory tests, these compounds were, however, active as mycelial growth retarders. β-Oxidation of the indole derivatives was observed in the liquid medium as well as in the soil.

Résumé Certaines concentrations de sodium et 1-S-n-octylisothiouronium indoleacétates, indolepropionates, indolebutyrates, phenoxy-, p-chlorophenoxy-, p-bromophenoxy-, et 2:4-bichlorophenoxyacétates pouvaient protéger le coton, le chanvre et les plants de tomates contre les fongi du sol dans la terre. Ceci pourrait être dû à de légers dérangements physiologiques des tissus végétaux. Au cours des épreuves en laboratoire, cependant, ces composés ont eu une action retardatrice sur la croissance mycélienne. Sur le milieu liquide aussi bien que dans le sol on a observé une β-Oxydation des dérivés de l'indole.

INTRODUCTION

Varying the natural hormone level in the plant has been of great value in agriculture. This paper represents an attempt to extend the practice to a physiological prevention of invasion of the plant roots by harmful soil fungi.

The effect of different natural and synthetic plant growth regulators on the pathogenicity of soil fungi has been reported (McClellan and Stuart, 1944; Jackston, 1946; Crowdy and Wain, 1950, 1951). Various auxins were of no effect in soil on cotton seedling diseases (Ranney and Bird, 1953; Gayed and Mostafa, 1961). Millikan and Fields (1964) found that 100 ppm of certain growth regulators reduced the growth of Pythium sp (93%), Rhizoctonia sp (56%), Fusarium oxysporum (75%). Another worker has recorded similar findings (Schwinn, 1965). On the other hand the application of auxins might increase the susceptibility of the plants to pathogenic fungi (Ellison and Cunningham, 1953; Croxall et al, 1957).

METHOD AND MATERIALS

A. Compounds

Acids: Indoleacetic (I), indolepropionic (II), indolebutyric (III), phenoxy- (IV), 4-chlorophenoxy- (V), 4-bromophenoxy- (VI) and 2:4-dichlorophenoxyacetic (VII) acids, HBr (hydrobromic acid).

(The roman numerals in brackets refer to the abbreviations used below).

Cations: Na, 1-S-n-octylisothiouronium (abbreviated to OT below).

The salts were dissolved in sterilized distilled water to give final concentrations of M/1,000, M/5,000, M/10,000, M/50,000, M/100,000, M/1,000,000 and M/10,000,000.

B. Fungicidal tests

Fusarium oxysporum (isolated from infected cotton, flax and tomato seedlings), Fusarium solani (isolated from infected tomato seedlings), Rhizoctonia solani (isolated from infected cotton, flax and tomato seedlings), Pythium sp (isolated from infected flax and tomato seedlings) were used as test fungi. Pythium sp was grown on PDA medium and the Fusaria on mineral-sucrose-agar medium in test tubes for 7-10 days. Spore suspensions were prepared as recorded by El-Nawawy and Tag El-Din (1967) and adjusted to 10.000 spores/ml. Thiamin solution (1 mg/ml, 20 ml) was added to the spore suspension of Pythium sp. Rhizoctonia solani was grown on mineral sucrose agar medium in a petri dish for 9-10 days.

The laboratory fungicidal test was done in mineral-sucrose medium buffered by monohydrogen phosphate-citric acid solution (El-Nawawy and Tag El-Din, 1967). The volume of solution in each test tube was 10 ml. The inoculum was 1 ml of the spore suspension, or a 4mm disc of mycelial growth of R solani. The results of fungicidal tests were recorded as the minimum time required for the earliest perceptible growth and the time required for complete disc formation - recorded by the naked eye.

The fungicidal test using the seedlings was done in a sandy loam soil (210 gm) in plastic pots (8 cm top diameter and 8 cm in depth). The soil in the pots was inoculated with a spore suspension (El-Nawawy and Tag El-Din, 1967) of the spore forming fungi, or with crushed mycelium of R solani. After watering the pots were incubated for seven days, and planted with cotton, tomato or flax. The solution of each compound was added (30 ml from below and 20 ml from above). Three replicates were made. The seedlings were examined and the number of dead seedlings recorded. The results were statistically analysed. Treated and untreated seedlings were cross sectioned, stained and examined in the usual way.

RESULTS

A. Representative data obtained from the fungicidal tests in liquid medium are recorded in Table 1. The following points can be observed: (a) M/1000 of all the (OT) salts were lethal to all fungi while the (Na) salts were not. (b) The three strains of Fusarium oxysporum differed in their response to the compounds; the strain isolated from tomato plants was the most sensitive especially to (I, Na) and (III, Na); the flax strain was also affected by (I, Na). (c) At M/1,000 (I, Na) was very active against R solani (from cotton) but (II, Na) was moderately active; the flax was moderately affected by (I, Na), (II, Na) and (IV, Na), but strongly inhibited by (V, Na), (VI, Na) and (VII, Na). The tomato strain was quite tolerant to the sodium salts of the two groups of plant growth regulators. At M/1,000, the (OT)

salts of all the acids were very toxic to R solani (from cotton or tomato seedlings), whilst at M/5,000 (I, OT) and (V, OT) were still very active against R solani (from cotton). (d) Pythium sp (from flax and tomato) were very susceptible to the (I, Na), (II, Na), (III, Na) and (VII, Na). (f) In many cases, although initial perceptible growth was delayed to some extent, the time required for the completion of the hyphal disc was fairly long. This effect was observed with all fungi treated with M/1000 of all the sodium salts; an effect with some continued at the lower concentration (M/50,000), especially (III, Na), (IV, Na), (V, Na) and (VII, Na) in the case of R solani. (e) No morphological changes were observed in the mycelial growth of treated F oxysporum (from tomato plants) and R solani (from cotton plants).

Table 1

Time in hours, required for the earliest perceptible growth (A) and complete disc formation of fungal growth (B) in the presence of several compounds in mineral-sucrose at pH 7

Compounds	0		M/1,000		M/5,000		M/10,000		M/50,000		M/100,000	
	A	B	A	B	A	B	A	B	A	B	A	B
Control	24	168	F oxysporum (from infected flax plants)									
I, Na			216	312	48	240	24	240	24	240	24	240
II, Na			24	264	24	216	24	264	24	144	24	144
III, Na			24	288	24	240	24	144	24	144	24	144
IV, Na			24	264	24	264	24	264	24	168	24	264
V, Na			24	264	24	264	24	144	24	144	24	144
VI, Na			24	264	24	144	24	264	24	264	24	264
VII, Na			24	312	24	312	24	312	24	312	24	216
Control	48	336	R solani (from infected flax plants)									
I, Na			72	336	72	336	48	312	48	336	48	336
II, Na			96	336	72	336	168	336	168	336	48	336
III, Na			72	336	48	336	48	264	72	336	96	336
IV, Na			48	336	72	336	48	288	72	264	72	336
V, Na			240	336	240	336	144	336	240	336	240	336
VI, Na			216	336	216	336	216	336	216	336	192	336
VII, Na			240	336	240	336	240	336	144	336	120	336
Control	24	240	F oxysporum (from infected tomato plants)									
I, Na			120	360	48	360	48	360	48	360	48	360
II, Na			48	360	48	360	48	360	48	360	48	360
III, Na			96	360	48	360	22	360	24	312	24	312
IV, Na			48	360	48	312	48	312	24	312	24	312
V, Na			48	264	48	312	24	312	24	312	24	312
VI, Na			48	312	48	312	24	312	24	312	24	312
VII, Na			48	360	48	312	48	312	48	312	48	312
Control	24	216	Pythium sp (from infected flax plants)									
I, Na			96	312	72	312	72	288	72	288	72	264
II, Na			96	312	96	312	96	312	48	264	48	264
III, Na			96	312	96	312	72	240	72	240	48	240
IV, Na			96	312	96	288	72	288	72	288	72	288
V, Na			120	312	96	312	96	288	96	288	72	264

Table 1 (cont'd)

Compounds	0		M/1,000		M/5,000		M/10,000		M/50,000		M/100,000	
	A	B	A	B	A	B	A	B	A	B	A	B
VI, Na			48	216	48	216	48	216	24	192	24	192
VII, Na			96	312	96	312	72	312	72	312	48	312
Control	24	216	Pythium sp (from infected tomato plants)									
I, Na			48	264	48	240	48	240	48	216	24	216
II, Na			24	240	24	240	24	216	24	216	24	216
III, Na			24	240	24	216	24	216	24	216	24	192
IV, Na			24	216	24	192	24	192	24	192	24	192
V, Na			24	216	24	192	24	192	24	192	24	192
VI, Na			24	192	24	192	24	192	24	192	24	192
VII, Na			24	216	24	192	24	192	24	192	24	192

Compounds	0		M/1,000		M/5,000		M/10,000		M/100,000		M/1,000,000	
	A	B	A	B	A	B	A	B	A	B	A	B
Control	24	192	F oxysporum (from infected cotton plants)									
I, Na			24	312	24	312	24	216	24	192	24	216
II, Na			48	312	24	216	24	216	24	192	24	192
III, Na			72	312	24	216	24	216	24	216	24	216
IV, Na			24	216	24	216	24	216	24	192	24	192
V, Na			24	216	24	216	24	216	24	192	24	192
VI, Na			48	216	24	216	24	216	24	192	24	192
VII, Na			24	216	24	216	24	216	24	192	24	192
Br, OT			-	-	572	792	24	264	24	216	24	192
I, OT			-	-	216	720	24	264	24	216	24	192
II, OT			-	-	168	576	24	264	24	216	24	192
III, OT			-	-	216	552	24	312	24	216	24	192
IV, OT			-	-	168	600	24	216	48	192	24	192
V, OT			-	-	48	552	24	264	24	216	24	192
VI, OT			-	-	168	672	24	216	24	216	24	192
VII, OT			-	-	24	360	24	216	24	216	24	192
Control	24	120	F solani (from infected tomato plants)									
I, Na			48	192	24	192	24	192	24	168	24	192
II, Na			24	192	24	192	24	192	24	192	24	192
III, Na			72	288	48	264	24	216	24	216	24	168
IV, Na			24	144	24	144	24	144	24	144	24	144
V, Na			24	264	24	240	24	240	24	240	24	240
VI, Na			24	264	24	264	24	240	24	216	24	216
VII, Na			48	216	24	192	24	192	24	192	24	192
Br, OT			-	-	120	336	48	312	24	240	24	168
I, OT			-	-	96	264	48	240	24	216	24	144
II, OT			-	-	96	288	24	288	24	192	24	144
III, OT			-	-	96	312	48	216	24	144	24	144
IV, OT			-	-	72	312	48	312	24	216	24	144
V, OT			-	-	72	288	48	240	24	192	24	192

Table 1 (cont'd)

Compounds	0	M/1,000		M/5,000		M/10,000		M/100,000		M/1,000,000	
	A										
VI, OT		-	-	96	312	48	312	48	240	24	144
VII, OT		-	-	96	312	48	264	24	192	24	168
Control	24	192		R solani (from infected cotton plants)							
I, Na		192	360	72	360	72	336	72	264	24	240
II, Na		72	336	72	336	24	240	24	240	24	240
III, Na		48	336	48	336	48	288	48	240	24	240
IV, Na		48	288	48	264	48	264	24	240	24	240
V, Na		48	288	24	264	24	264	24	240	24	240
VI, Na		24	288	24	264	24	264	24	264	48	240
VII, Na		24	312	24	288	48	240	24	240	24	240
Br, OT		-	-	168	696	120	360	24	216	24	192
I, OT		-	-	408	792	192	360	72	360	72	312
II, OT		-	-	264	720	216	360	24	192	24	192
III, OT		-	-	264	648	120	360	24	216	24	192
IV, OT		-	-	336	648	192	360	24	216	24	192
V, OT		-	-	240	528	144	360	24	240	24	240
VI, OT		-	-	408	840	144	360	24	264	24	264
VII, OT		-	-	72	480	72	312	72	264	24	264
Control	24	168		R solani (from infected tomato plants)							
I, Na		24	288	24	216	24	168	24	168	24	168
II, Na		24	216	24	168	24	168	24	168	24	168
III, Na		24	216	24	168	24	168	24	168	24	168
IV, Na		24	216	24	168	24	168	24	168	24	168
V, Na		48	216	48	168	48	168	24	168	24	168
VI, Na		48	264	24	192	24	168	24	168	24	168
VII, Na		48	206	48	168	48	168	48	168	24	168
Br, OT		-	-	312	590	96	312	24	168	48	168
I, OT		-	-	240	590	96	312	24	168	24	168
II, OT		-	-	190	552	72	192	24	168	24	168
III, OT		-	-	192	504	72	264	24	168	24	168
IV, OT		-	-	240	504	96	312	48	168	24	168
V, OT		-	-	240	576	72	312	24	168	24	168
VI, OT		-	-	216	504	72	264	24	168	24	168
VII, OT		-	-	144	336	48	264	24	168	24	168

B. Combined Phytocidal-Fungicidal Effects

Cotton plants: (Table 2) A significant increase in the mean percentage mortality of the seedlings in the presence of R solani plus one of the compounds as compared with that resulting from the compounds alone was observed in the presence of certain concentrations of (Na) and (OT) salts of I, II and VII. On the other hand, most of the compounds at most concentrations gave a highly significant reduction in the mean percentage mortality of cotton seedlings caused by R solani. The most effective in this respect were (a) (VI, Na), (at M/10,000 and M/100,000), (b) (VI, Na) and (IV, Na) 4-bromo-, and phenoxy- (at M/1000), (c) (I, OT) (at M/1,000,000), (IV, Na M/10,000,000).

The combined effect of the compounds plus F oxysporum lead to a significant reduction in the mean percentage mortality as compared with that observed in the infected soil. The exceptions were (a) (IV, Na), (VI, Na) and (OT) salts of VI and VII at M/1000 and (b) (V, Na) and (VII, OT) at M/10,000.

The most effective compounds in reducing the percentage mortality caused by F oxysporum were (a) (II, Na) (at M/10,000 and M/10,000,000), (b) (IV, Na) and (V, Na) (at M/10,000,000), (c) (IV, Na) (at M/10,000 and M/10,000,000), (I, OT), (at M/1000), (VI, Na) (at M/10,000 and M/100,000) and (II, Na) (at M/1,000,000).

Flax plants: (Table 3) The addition of the compounds caused a decrease in the mean percentage mortality caused by F oxysporum. On the other hand, most of the compounds caused a significant decrease in the mean percentage mortality caused by Pythium sp (I, Na), (II, Na) at M/10,000 and (VI, Na) at M/100,000 were not able to overcome the effect of the fungus.

Table 2

Modified means of percentage mortality of 16 days old cotton plants grown in sandy loam soil infected (A) or non-infected (B) with certain fungi in the presence of several compounds

Compounds	M/1,000		M/10,000		M/100,000		M/1,000,000		M/10,000,000	
	A	B	A	B	A	B	A	B	A	B
(a) R solani (from infected cotton plants)										
I, Na	4.7	36.7**	24.0	13.8**	16.7	29.7**	17.5	28.7**	14.9	14.5*
II, Na	22.5	24.6**	19.8	22.9**	12.1	36.2**	4.7	11.8**	0	21.2*
III, Na	15.4	23.9**	5.5	5.1**	13.2	7.3**	6.1	12.9**	13.2	15.7*
IV, Na	12.6	16.2**	16.6	16.9**	21.3	9.0**	25.1	15.4**	13.4	13.8*
V, Na	-	45.0*	12.7	25.6**	5.8	16.3**	13.2	17.2	13.2	13.2*
VI, Na	-	-	37.9**	41.6**	15.6	5.5**	12.1	24.6**	11.6	18.5*
VII, Na	-	-	36.9**	90.0	16.2	13.4**	10.8	23.0**	4.3	24.8*
Br, OT	20.6	24.2**	16.5	25.6**	44.3	24.0**	11.6	20.4**	18.9	22.3*
I, OT	13.2	14.8**	10.1	20.9**	7.3	26.1**	14.1	5.1**	13.6	22.6*
II, OT	16.4	21.1**	16.1	28.2**	5.1	16.0**	16.3	28.5**	7.3	26.7*
III, OT	9.6	36.8**	20.5	20.7**	24.1	37.3**	27.1	28.4**	19.8	20.3*
IV, OT	25.6	38.9**	27.1	21.3**	28.6*	36.6**	23.6	36.7**	17.0	35.2*
V, OT	-	-	45.0**	42.9**	25.8	36.5**	16.7	32.3**	21.6	21.1*
VI, OT	-	-	27.2	35.4**	12.3	30.4**	14.5	30.7**	20.0	23.4*
VII, OT	-	-	55.2**	25.8**	8.1	31.9**	29.9*	24.1**	9.8	21.4*
(b) F oxysporum (from infected cotton plants)										
I, Na	4.7	38.2*	24.0	19.0**	16.7	19.4**	17.5	30.1**	14.9	23.6*
II, Na	22.5	25.4**	19.8	5.1**	12.1	16.4**	4.7	23.3**	0	4.7*
III, Na	15.4	20.8**	5.5	16.0**	13.2	23.9**	6.1	28.8**	13.2	14.6*
IV, Na	12.6	18.0**	18.6	14.5**	21.3	8.9**	25.1	10.9**	13.4	6.5*
V, Na	-	-	12.7	90.0	5.8	18.9**	13.2	13.1**	13.2	6.5*
VI, Na	-	90.0	37.9**	21.3**	15.6	22.8**	12.1	27.9**	11.6	24.0*
VII, Na	-	22.5**	38.9**	36.7*	16.2	36.5*	10.8	15.4**	4.3	15.9*
Br, OT	20.6	27.8**	16.5	14.4**	4.3	17.7**	11.6	31.3**	18.9	16.0*
I, OT	13.2	10.9**	10.1	21.0**	7.3	23.8**	14.1	19.3**	13.6	22.2*
II, OT	18.4	33.8*	16.1	33.2**	5.1	21.9**	18.3	10.9**	7.3	23.3*
III, OT	9.6	27.7**	20.5	25.1**	24.1	38.3*	27.1	27.7**	19.0	23.6*
IV, OT	25.6	21.6**	27.1	12.1**	28.6*	12.1**	23.6	19.7**	17.0	16.7*

Table 2 (cont'd)

Compounds	M/1,000		M/10,000		M/100,000		M/1,000,000		M/10,000,000	
	A	B	A	B	A	B	A	B	A	B
V, OT	-	-	45.0**	33.0**	25.8	23.8**	16.7	24.7**	21.6	22.7*
VI, OT	-	-	27.2	27.2**	12.3	20.1**	14.5	21.3**	20.0	29.0*
VII, OT	-	-	55.2**	42.9	8.1	25.0**	29.9*	17.6**	9.8	24.2*

* at P = 0.5 ** at P = 0.01

Mean percent mortality of cotton seedlings in non-infected soil = 11.6; in soil infected with R solani = 65.3; in soil infected with F oxysporum = 58.0 in the absence of the compounds.

Tomato plants: Most of the compounds produced an increase in the number of healthy plants in the presence of R solani. The most active in this respect were (a) (I, Na), (III, Na) and (VII, Na) at M/10,000, (b) (V, Na) (at M/100,000).

Table 3

Modified means of percentage mortality of 16 days old flax plants grown in infected loam (A) or (B) non-infected loam in the presence of several compounds

Compounds	M/10,000		M/100,000		M/1,000,000	
	A	B	A	B	A	B
	F oxysporum (from infected flax plants)					
I, Na	16.2	35.6**	13.9	27.1**	33.7	43.8**
II, Na	10.5	28.4**	12.1	33.7**	10.9	39.4**
III, Na	13.8	40.0**	10.0**	38.0**	5.4	47.9**
IV, Na	8.9	40.5**	10.3	39.4**	7.2	32.9**
V, Na	8.3	- **	6.3	12.6**	10.1	44.9**
VI, Na	14.9	35.0**	8.1	47.1**	7.8	32.7**
VII, Na	9.7	54.5**	10.9	21.0**	7.9	34.8**
	Pythium sp (from infected flax plants)					
I, Na	16.2	64.2	13.9	28.1**	13.7	48.3**
II, Na	10.5	68.2	12.1	40.3**	10.9	38.7**
III, Na	13.8	47.1**	10.8	52.2**	5.4	32.3**
IV, Na	8.9	48.1**	10.3	44.1**	7.2	54.1**
V, Na	8.3	- **	6.3	46.5**	10.1	52.5**
VI, Na	14.9	47.9**	8.1	64.1	7.8	58.4**
VII, Na	9.7	18.9**	10.9	11.2**	7.9	29.5**

* at P = 0.5 ** at P = 0.01

When none of the compounds were used the mean percentage mortality of flax seedlings in non-infected soil = 6.9; in soil infected with F oxysporum = 80.3; in soil infected with Pythium sp = 76.9.

None of the compounds were able to protect tomato seedlings against Pythium sp at M/10,000 but (VI, Na) and (VII, Na) at M/100,000; (I, Na) and (VII, Na) at M/1,000,000, gave a good control of Pythium sp.

c. Histological Studies

Transverse sections of the root of the seedlings showed that R solani alone or in the presence of M/1,000,000 of (II, Na) caused a darkening in the tissues and disintegration of the cortex on one side; the symptoms being more obvious in cotton than with flax and tomato seedlings grown in loamy soil. In the presence of (III, Na), (VI, Na) and (VII, Na), at M/100,000 the symptoms disappeared. (IV, Na), (V, Na) were moderately active against R solani.

DISCUSSION

The sodium salts of the indolyl and aryloxy derivatives proved to be active as retarders of mycelial growth. Apart from a few cases the initial growth was not seriously inhibited or delayed, but the time required for complete disc formation was significantly longer. The most sensitive fungus in this respect was R solani. The isothiouronium salts of the same acid were strongly fungitoxic at higher concentrations. At lower strengths they could not inhibit initial growth of the fungi, but the time required for complete disc formation was significantly longer. Hence some of these compounds were good inhibitors of the mycelial growth of certain fungi. Moreover, the isothiouronium cation caused a marked increase in the fungitoxicity of the molecule, due possibly to the combined effect of the cation and anion.

The mechanism of invasion of the plant by such fungi might depend upon certain natural physiological conditions of the tissues of host plants. The upsetting of such conditions might reduce the pathogenicity of these fungi. If so, this idea could provide a new method for an indirect control of pathogenic soil fungi. El-Nawawy et al (1969) were able to prove that symbiosis between the Rhizobium bacterium and horse beans (Vicia fabae) can be prevented by a few ppm of certain growth-regulators. The production of excess auxins in diseased plants (Pilet, 1953) might be responsible for the one sided invasion of roots of cotton seedlings by R solani and prevent a further invasion because of the increase of IAA content.

From the results, the protection of cotton seedlings against F oxysporum was obtained with all the compounds at a concentration of M/10,000 except sodium and 1-S-n-octylisothiouronium p-bromo-, p-chloro-, and 2:4-dichlorophenoxyacetate. Protection might be afforded either through the direct effect against the fungus by the isothiouronium salts or through the unsuitable physiological condition of the tissues as a result of the sodium salts of the acids. M/10,000 of sodium p-chlorophenoxyacetate and the isothiouronium salts of p-bromo-, and 2:4-dichlorophenoxyacetates caused some promotion of fungal pathogenicity, perhaps through encouraging the fungus or by decreasing the plant tolerance. This agrees with the previous finding (Croxall et al 1957). With R solani mutual decrease of fungitoxicity and phytotoxicity was observed in the presence of M/10,000 1-S-n-octylisothiouronium 4-chlorophenoxyacetate. This might be due to consumption of the compound by the fungus or the plant being enriched with a plant-growth-regulator, might resist attack by the fungus. At M/100,00 all the compounds, except sodium 4-chloro-, and 2:4-dichlorophenoxyacetates and, at M/1,000,000 all the compounds provided complete protection against R solani.

F oxysporum increased the phytotoxic effect of M/1,000 of sodium 4-chloro- and 2:4-dichlorophenoxyacetates and decreased the phytotoxic properties of the isothiouronium salt of the former anion. On the other hand, R solani increased the phytotoxicity of sodium 2:4-dichlorophenoxyacetates and decreased that of its isothiouronium salt. Thus the interaction of the two fungi with the compounds was not the same.

The sodium salts of the three indole derivatives protected flax seedlings against F oxysporum. Sodium phenoxyacetate was able to give a similar protection against R solani. Similar protection of flax seedlings against Pythium sp was obtained with the sodium salts of aryloxyacetic acids and the indole derivatives at different concentrations.

M/1,000 of 1-S-n-octylisothiouronium bromide and indoleacetate protected tomato seedlings against F solani. Sodium indolebutyrate behaved similarly but at lower concentrations.

Sections in the treated roots of cotton seedlings indicated that the β-oxidation might have played a role with the three indole derivatives. Sodium indoleacetate and indolebutyrate were active against the fungus while sodium indolepropionate was not. The responsible enzyme may have originated from the plant on the fungus. Taylor and Wain 1963, established β-oxidation by micro-organisms.

In conclusion a "new method" for the indirect control of soil fungi by applying very low concentrations of physiologically active compounds has been revealed.

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THE OCCURRENCE OF PYRENOCHAETA LYCOPERSICI ON LETTUCE

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Summary. Studies carried out at Luddington Experimental Horticulture Station by Last & Ebben (unpublished) suggested that lower yields of lettuce occurred in plots which had carried a tomato crop showing much brown root rot (BRR) than when attacks were less severe.

It has not proved possible to obtain a reliable assessment of infection of lettuce plants by visual inspection of roots or by isolation of fungi from discoloured roots. An estimation of incidence of root infection can, however, be obtained by isolation of Pyrenochaeta lycopersici Schneider & Gerlach from a substantial sample of the root system of plants. Using this basis the fungus has been shown to occur more frequently on roots in soil regarded as heavily infested and in plots giving lower lettuce yields. The pathogenicity of P. lycopersici to lettuce has been established but the experiments made have not produced direct evidence that infection is accompanied by a reduction in yield, although the possibility that this occurs cannot be disregarded.

Résumé. Les études poursuivies par Last & Ebben (non publicées) a la Station Experimentale d'Horticulture de Luddington ont suggérées que les rendements faibles de laitues apparaissent plutôt dans les parcelles ayant porté les tomates manifestant beaucoup de symptôms de pourritures brunes de racines que dans celles où la pourriture des tomates est moins importante.

Il n'a pas été possible d'obtenir une bonne estimation du degré de contamination des laitues par un examen visual des racines ou par isolement des champignons dans les racines décoloreés. Cependant une estimation de l'incidence du degré d'infection des racines peut être obtenue par isolement du Pyrenochaeta lycopersici Schneider et Gerlach à partir d'un échantillon assez important du systeme racinaire des plantes. Par cette méthode, le champignon a pu être isolé plus frequemment des racines provenant des sols fortement contaminés et des parcelles ayant donné des faibles rendements de laitues. La pathogénie de P. lycopersici sur la laitue a été etabliée mais les expériences qui ont été faites n'ont pas mis clairement en évidence que l'infection est accompagnée d'une réduction de rendement, bien qu' on ne puisse écarter cette possibilité.

INTRODUCTION

Brown root rot (BRR) and corky root symptoms on tomato have been familiar to growers for a long time, and severe attacks have been often associated with poor crops and losses in yield. A grey sterile fungus, first recorded on tomato roots in England by Williams (1929), has been shown to be a primary pathogen of tomatoes producing both BRR and corky root symptoms (Richardson & Berkeley, 1944; Termohlen, 1962; Last & Ebben, 1966). This fungus has now been named Pyrenochaeta lycopersici (Schneider & Gerlach, 1966).

The build-up of BRR in tomato crops for the first five successive years in untreated soil at Luddington Experimental Horticulture Station was studied by Last et al (1969). In this work the possibility was examined of predicting the point at which soil treatment by steam or chemicals becomes economically justifiable. The question arose as to whether lettuce, an alternate crop between the tomato crops, was affected by P. lycopersici; whether yield and quality of the lettuce crop were affected, or whether a build-up of inoculum on lettuce roots would affect predictions of BRR in subsequent tomato crops.

In inoculation experiments, Termohlen (1962) re-isolated the "corky root" fungus from a wide variety of hosts including lettuce, and Last (1969) stated that P. lycopersici occurred on lettuce roots. However, the significance of this pathogen on lettuce remains in doubt. The present work was undertaken to ascertain if P. lycopersici infects lettuce and if so how severe are the attacks.

EXPERIMENTAL

Symptoms on lettuce roots

Roots of lettuce from untreated plots at Luddington, shown to have high levels of P. lycopersici infestation because of attacks on tomato, were compared with roots from plots treated with methyl bromide to minimise levels of the pathogen. Roots from both sources showed discoloration which consisted mostly of light yellow patches up to 1 cm long but which occasionally graded through to shades of brown. The discoloured patches were mostly superficial. In 1969 there was little consistency in the fungal species isolated from tissues showing these symptoms. The standard method of isolation employed was to wash roots in running water to remove soil, surface sterilise in sodium hypochlorite solution (1.5% available chlorine) for 0.5 to 1 min and rinse in three changes of sterile water. A transverse sample of 7 mm root segments was taken with a sterile scalpel and forceps 6 cm below the top of the root system. The segments were plated out in warm agar (approximately 20 segments per plate) as in dilution plating techniques, and incubated at 24°C. The medium employed was 3% malt extract agar containing aureomycin (50 ppm).

In 1970, mainly at harvest, visual estimates were made of the size of discoloured patches on affected root areas on plants from untreated plots and from those treated with methyl bromide. Five or six plants were examined from each plot. On each root system the

numbers of lesions falling into the following categories were counted:- (1) smaller than 0.5 cm, (2) 0.5 to 1.0 cm, and (3) larger than 1.0 cm. A disease index was calculated by allocating one point, two points, and three points to each category respectively and determining the total for each plot and the average per plant. The disease index was not consistently higher for plants from untreated plots when compared with that for plots treated with methyl bromide during the preceding one or two years. Moreover, the disease index did not match the levels of isolation of P. lycopersici from the roots from the same plots.

Estimation of incidence of root infection by isolation of fungus

Although P. lycopersici was rarely isolated from the yellow coloured patches on roots it was consistently isolated from lettuce roots grown in infested soil. Examined under a stereoscopic microscope these infected roots bore a number of small brown lesions and rotted rootlet ends. Such symptoms, however, were seen almost as frequently on roots from plots treated with methyl bromide as from untreated plots, although a much greater proportion of lesions from roots from untreated plots yielded P. lycopersici. This organism was frequently isolated from root segments which showed no macroscopically visable symptoms, and so it was considered necessary to make isolations from root samples to obtain a quantitative estimate of the incidence of infection. Isolations were made from various samples over a three year period at the times stated: 1969, at harvest; 1970, 3 weeks before harvest and at harvest; 1971, 5 and 3 weeks before harvest and at harvest. In 1969 and 1970 four to six plants per plot from 6 plots were used, but in 1971 six plants from each of 16 plots were included. In 1969 half of each plot was planted with cv. May Princess and the other half with cv. Profos and equal numbers of plants of each cultivar per plot were examined. In 1970 and 1971 only cv. May Princess was planted. In 1969 and 1970 new and old roots were examined separately. The plots sampled were selected to enable comparisons to be made between low and high levels of soil infestation with P. lycopersici, as rated in 1967 by level of BRR in the previous tomato crops, and between these levels of soil infestation as expected according to the soil treatments with methyl bromide given in 1968, 1969 and 1970.

During examination of plates it soon became apparent that more than one species of fungus produced grey mycelium similar to that of P. lycopersici. Some of the isolates grew faster than others and produced pycnidia typical of Phoma, and so were excluded from counts of P. lycopersici made in 1969. Some of these isolates producing pycnidia were subsequently identified as Phoma chrysanthemicola Hollos, and proved to be pathogenic to lettuce causing extensive root infection. In 1970 it was realised that although there were isolates typical of Pyrenochaeta lycopersici or of Phoma chrysanthemicola a considerable number were intermediate between the two species. These intermediates were tentatively included with the typical isolates of P. lycopersici in 1970. It was possible in 1971 to determine the identify of these intermediates by means of a rapid pathogenicity test which

will be described elsewhere. The isolation plates were therefore held at 4°C until the identity of these intermediates was determined. The pathogenicity test also showed that in 1970 the intermediates, tentatively allocated to P. lycopersici, could in the great majority of cases be properly referred to that species.

P. lycopersici was isolated with slightly greater frequency from roots of cv. Profos than from cv. May Princess. It was frequently isolated from both young and old roots, but there was no consistency in the results for comparative frequency. There was usually an increase in the frequency with which P. lycopersici was isolated from roots as the season progressed towards harvest.

The plots had been grouped in 1967 into those heavily infested and those lightly infested according to the levels of BRR present in the previous tomato crops. It was therefore possible to compare the frequency with which P. lycopersici was isolated in each of the three years from lettuce roots grown in soil referred to each of these groups. It should also be borne in mind that many of the plots grouped as lightly infested in 1967 had more recently been treated with methyl bromide. The results obtained showed that, in general, P. lycopersici was isolated more frequently from roots grown in the heavily infested soil. Also, in general, higher lettuce yields were associated with the lower numbers of successful isolations of P. lycopersici from roots grown in lightly infested plots and vice versa in heavily infested plots. In 1970 and 1971 it was found that, in general, there was an association of higher yields of lettuce with the lower numbers of successful isolation of P. lycopersici from plants grown in plots treated with methyl bromide. In these instances the possibility that plant growth may have been influenced by treatment with methyl bromide other than by the effects of the treatment on this pathogen cannot be eliminated.

Pathogenicity of P. lycopersici to lettuce and its effects on yield

At Luddington the experimental programme from 1965 onwards involved the growing of spring maturing lettuce between tomato crops. From the first year of planting there was evidence that soil sterilisation treatments had an effect on lettuce, inducing better growth of roots and foliage with a higher proportion of plants in the top grade and fewer discarded as commercially unacceptable, as compared with those in untreated soil. The BRR levels recorded for the tomatoes preceding the first lettuce crop were as high as 33.7% for the untreated control plots when determinations were made at plant maturity.

Further evidence regarding the possible effects of P. lycopersici on yield of lettuce in later seasons at Luddington is available. As has been stated already, the experimental plots were divided in 1967 into two groups having high and low levels of BRR in the previous tomato crops. Some plots in each group were treated in autumn 1967 with methyl bromide at one of two levels. The yields of lettuce from each plot in spring 1968 were recorded (Table 1). The mean yield from untreated plots classed as heavily infested was lower than that from plots classed as lightly infested. Moreover, in the heavily infested plots the higher rate of treatment was necessary to raise the yield to the level achieved by the lower treatment rate in lightly infested soil.

Table 1

Effects of soil treatment on mean yields of spring lettuce 1968

Treatment	Yield of marketable produce (lb/plot)	
	1967 BRR level	
	High	Low
Untreated	40.2	48.4
Methyl bromide in autumn, 1967 0.5 lb/100 ft^2	49.4	53.2
Methyl bromide in autumn, 1967 1.0 lb/100 ft^2	53.0	54.9

Because of this circumstantial evidence of the possible effect of P. lycopersici on yield of lettuce it was desirable to establish the pathogenicity of this organism to lettuce, and to examine effects on yield when inoculated and uninoculated plants were compared. Lettuce seedlings, cv. Profos, were therefore inoculated with isolates of P. lycopersici obtained from roots of lettuce and tomato. Inoculum was prepared by growing each isolate in a damp mixture of cornmeal and sand (cornmeal 2.5 g : sand 97.5 g) at room temperature until the mixture was thoroughly permeated by the fungus. The inoculum was then thoroughly mixed with soil at the rate of 1 : 25 by volume and placed in pots of 13 cm diameter. One seedling grown in Levington compost in "Jiffy" pots was then planted in each pot. Control plants were provided in soil to which sterile sand-cornmeal medium was added at the appropriate rate, as well as those growing in unamended and uninoculated soil. Experimental plants were maintained under glass at 17°C for 10 weeks. P. lycopersici was isolated much more frequently from lettuce roots inoculated with the isolate from lettuce as compared with those inoculated with the tomato isolate. The lettuce plants inoculated with either isolate showed no reduction in the mean dry weight of foliage as compared with either of the sets of control plants when examined 10 weeks after inoculation. In a similar experiment no reduction in the mean dry weight of tomato plants (cv. Potentate) inoculated with P. lycopersici occurred at 10 weeks or 19 weeks after inoculation.

Two further experiments were made in the open air at the Botany Experimental Grounds situated at Jodrell Bank, Cheshire, and Fallowfield, Manchester, in 1969 and 1970 respectively. In 1969 cv. Profos and in 1970 cv. May King were used. In each year plants inoculated with P. lycopersici were compared with those which were uninoculated. Each treatment was replicated four times in a randomised block lay-out with 50 plants in each plot, plants being 20 cm apart. Guard rows of lettuce separated the experimental plots.

In 1969 inoculations were made by a method similar to that described by Last & Ebben (1966) using sterilised wheat seeds inoculated with the fungus. Seedlings were planted in dibble holes

in each of which was placed 140 ml of an inoculum mixture (1 part inoculum : 7 parts soil by volume) or similar quantities of a mixture of sterilised wheat seeds and soil. Eight weeks after planting the mean fresh weight of plants in the control plots was lower than that for the inoculated plots, but a concurrent glasshouse experiment showed that the addition of sterilised wheat seed to soil reduced plant growth. It was also noted in the field that the fungus did not infect roots beyond the originally contaminated soil.

In the 1970 experiment vermiculite was used as the inoculum base after preliminary trials in a glasshouse. Sterilised vermiculite soaked in Czapek Dox solution was inoculated with the pathogen and incubated until the fungus had spread through it. It was then flushed with running water to free it of nutrients and mixed with soil at the rate of 1 part to 20 parts soil, this being the lowest ratio which provided effective infection in glasshouse tests. The mixture was then placed in holes in the plots using 570 ml per hole and a lettuce seedling planted in the centre of the mixture. Few isolations of P. lycopersici were made from roots of inoculated plants at 4 or 8 weeks after planting. At the latter time slight infection by the pathogen was recorded on 30% of the inoculated plants as compared with 20% of the uninoculated plants. There was no evidence that inoculation had decreased the fresh weight of the plants. As with naturally infected plants no macroscopically visible symptoms of infection were observed on any of the roots of plants inoculated with P. lycopersici. The fungus did, however, cause small brown lesions and rotted ends of rootlets that were observable under the microscope.

DISCUSSION

It is clear that P. lycopersici was frequently present on roots of lettuce at the one centre where observations were made. The pathogenicity of this fungus to lettuce has been established. It has, however, proved difficult to obtain direct evidence of an effect on lettuce growth that is caused by infection with P. lycopersici. Because infection caused no distinctive symptoms on lettuce roots it was necessary to obtain a disease index by isolating the pathogen from samples of roots and recording the proportion of roots yielding it. This procedure differed from visual methods of assessment by reflecting the incidence of individual points of infection rather than the severity of the disease.

High incidence of isolation of P. lycopersici from lettuce roots was associated with lower yields of lettuce in plots at Luddington untreated with methyl bromide, and this may suggest that this pathogen may have caused a reduction in yield. The experiments made in the field and in the glasshouse did not provide direct evidence to implicate P. lycopersici as a cause of yield reductions, but in spite of the results obtained such a possibility cannot be dismissed. The reasons for this conclusion are (1) that the small scale experiments may not have been adequate to establish yield differences, and (2) the use of inoculum in the experiments may not have emulated naturally infested soil. The evidence, however, does

suggest that there is no large scale effect.

The ability of P. lycopersici to infect lettuce roots indicates that lettuce may act as a carry-over crop for this fungus when it occurs between two tomato crops. Further information on the contribution of the infected lettuce roots to the inoculum potential will be published elsewhere.

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NOTES

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ENVIRONMENTAL REQUIREMENTS IN STORE AS DETERMINED BY POTENTIAL DETERIORATION

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Summary There are three classes of storage deterioration, caused respectively by microbial attack, evaporative loss, and biochemical change. All plant material suffers from all three types of loss, but the relative importance varies with the commodity. Commodities differ in their potential for storage, ranging from produce which is inherently ephemeral, either because of rapid biochemical change to an unacceptable state (e.g. pears, asparagus), or because of great susceptibility to microbial attack (e.g. raspberries, strawberries), or to wilting (e.g. lettuce); to, on the other hand, the natural overwintering or perennating organs which have a storage potential of several months (e.g. potatoes, white cabbage). The storage potential of a commodity can often be related to its function on the growing plant, and perennating organs can often best survive those environmental conditions under which they evolved in the ancestral home.

Storage conditions must frequently be a compromise between conflicting demands, the weight given to which must depend upon what is the most serious form of loss in the particular commodity. In general, and leaving aside chemical treatments, microbial attack is reduced by low temperature and low humidity; wilting by high humidity; and biochemical change by low temperature - though there are several exceptions and several commodities which are damaged by low temperatures - and in certain cases by controlled atmosphere storage.

INTRODUCTION

In assessing the quality of a stored fruit or vegetable the criteria of acceptability are ultimately based on a comparison with the characteristics of the commodity harvested at a stage optimal for consumption and eaten immediately. This stage may well be, and often is, ephemeral; and to retain it for a useful length of time in storage is a matter of some difficulty. Change in a living organism in storage is inevitable and it may be necessary to harvest before the optimum is reached and then store carefully to bring the produce to as near the optimum as possible at the time of sale.

All fruit and vegetables are subject to the same forms of storage deterioration, which we may classify broadly under the headings of: rotting caused by pathogens; water loss; and changes in composition and metabolism. The effects of this last may be manifested in several ways, from a direct influence on acceptability, nutritive value or safety; to changed physiological behaviour, as in the case of renewed growth of storage organs. Some change in composition is of course inevitable during the storage of living organisms which are perpetually losing carbon in the volatile products of respiration, but the change directly resulting from this may be of minor importance compared to the results of the

hundreds of syntheses, molecular re-arrangements and breakdowns which are also perpetually occurring.

Although all fruit and vegetables suffer from the same three categories of deterioration, the relative importance of these categories varies considerably from commodity to commodity. The first aim in storing any given crop, therefore, must be to reduce or eliminate the most important source of storage loss of that crop. It is quite possible that, in so doing, one provides conditions which enhance another form of deterioration - for example a high humidity may reduce water loss but enhance bacterial rotting - and a compromise must be sought in which losses due to all causes are kept to a minimum; but the first approximation to the desired storage environment is always that which concentrates on reducing the most important source of loss.

STORAGE POTENTIAL

Foodstuffs which are grouped together as vegetables are of very varied nature, including stems, leaves, buds, inflorescences and storage organs. Each of these structures may have storage potentialities or hazards which are inherent, in most cases, because the function it performs in the plant has necessitated the evolution of characters which also, perhaps incidentally, influence storage. Fruits, even though they have the same basic reproductive function, are very varied in structure and equally varied in storage potential.

Each category of foodstuff is discussed below with a view to determining its inherent storage characteristics.

A. Roots. The fibrous roots of plants have no features which render them attractive as human food and they have not been exploited as such. Swollen roots, which function as storage organs, are discussed under that heading.

B. Stems. The stem normally has primarily a support function which involves structural rigidity. The rapidly growing apex is not rigid, however, but derives some mechanical strength from turgor and, some distance behind the growing point, from cellulose thickening of the cell walls. Further back from the growing point reinforcement is usually introduced in the form of lignin. An unlignified stem, which usually but not necessarily implies the young tip of the stem, can be eaten, the principal European example being asparagus. If we include flower- and leaf-stalks in the category of stem, then such commodities as sprouting broccoli are largely composed of stems and celery almost wholly so. Stems which are harvested very young and unlignified, if they are growing very rapidly and are metabolically very active - as is the case with asparagus - will continue their development, including lignification, after harvest. The cut ends and the surfaces of such young shoots are also susceptible to rapid loss of water by evaporation, and their surface (cm^2)/weight (g) ratio is moderately high (c. 3-6:1). The immediate physiological hazards associated with the storage of edible stems are thus lignification and water loss.

C. Leaves. Leaves are typically photosynthetic organs and their structure has evolved in such a way as to provide the maximum illumination for the maximum number of cells (i.e. a thin lamina or a needle) and a surface which permits the rapid diffusion of carbon dioxide into the leaf. The rate of this may be of the order of 4×10^{-3}mg $cm^{-2}hr^{-1}Pa^{-1}$ (see footnote) averaged over both surfaces of a leaf with

Note For readers unfamiliar with the Systeme Internationale d'Unites (SI) it may be useful to define the Pascal (Pa). This is the SI unit of pressure and is 1kg $m^{-1}s^{-2}$. One millibar = 100 Pa.

open stomata. The coefficient of diffusion of water vapour is about 40% greater than that of CO_2, and this, coupled with a total surface (cm^2)/weight (g) ratio of perhaps 20-40:1[2] (though this varies considerably) indicates that water vapour could diffuse from such a leaf at a rate of about 1-2 x 10^{-1} mg $g^{-1}hr^{-1}Pa^{-1}$. The water vapour pressure deficit could well exceed 1000 Pa under ambient conditions in the summer, and in such an environment a leaf, because of the structure necessitated by its function as a photosynthetic organ, could, so long as its stomata remained open, lose water at a rate of about 10-20% of its weight per hour. Even with the stomata closed, a leaf could lose, by cuticular evaporation, because of its large surface/weight ratio, from a tenth to a fifth of this amount. Normal photosynthetic leaves, in which we may include spinach, kale, the looser forms of lettuce, the green parts of untrimmed leeks and spring onions, are thus inevitably subject above all to water loss, and the first requirement of storage is to reduce this. There are other sources of loss, including rotting by pathogens and deleterious biochemical changes, such as loss of chlorophyll, but these only become of importance when we have extended the storage life sufficiently, by reducing water loss, to give time for their development.

D. Buds. A bud typically consists of a growing point, surrounded and enclosed by young leaves, formed because the biochemical balance in the shoot has ceased to permit internode elongation and cell division. This swing in the balance will occur in a constant environment, though its course and velocity are much influenced by ambient conditions, and it is reversible, growth being resumed after an interval which we refer to as bud dormancy. In some plants the biochemical balance for much of the life history appears to be on the borderline between a state of growth permissive of expansion and one in which this cannot occur. We get leaf expansion but internode elongation is much reduced at an early stage and almost the whole plant, with leaves ranging from young unexpanded to fully expanded, takes the form of a bud, prior to a later swing to rapid elongation and flowering. The cabbage is an example. In the Brussels sprout the biochemical balance which normally holds axillary buds dormant and unexpanded appears to be marginal, with the result that we get much enlarged buds, or sometimes, if the balance swings too far, elongation and lignification as well.

The outer leaves of a bud, such as a cabbage or a Brussels sprout, are susceptible to water loss as described above under the heading of 'leaves'. The air in contact with the leaves forming the bulk of the bud is, however, almost saturated with water vapour, and hence with a negligible water vapour pressure deficit. The bulk of the bud thus cannot readily lose water by evaporation, and wilting is not such a problem as it is in the case of isolated leaves and looser structures. The longer life leads to forms of deterioration other than wilting, such as attack by pathogens, lignification, loss of chlorophyll and other unacceptable changes in composition - an example is the formation of strong-smelling isothiocyanate derivatives in Brussels sprouts - assuming greater importance. There is also the possibility, if storage is sufficiently prolonged, as in winter-storing cabbage, that the biochemical balance may swing to the next stage of development - elongation and flowering.

E. Inflorescences. The main examples of inflorescence with which we are concerned are cauliflowers, broccoli, sprouting broccoli and calabrese. In a form acceptable for consumption these consist of the unopened flower buds, the unlignified stems on which these are borne, and a variable amount of leafy material, stems forming the largest proportion by weight - usually about 50-60%.

The immature inflorescence is ephemeral; very soon the florets open and the flower stalks elongate and lignify. In storage we are concerned with avoiding this natural progression to an unacceptable state, and also with avoiding other causes of unacceptability or loss - for example wilting and chlorophyll loss of the leafy material associated with the inflorescence, discoloration of the curd of cauliflowers and broccoli following mechanical damage, and attack by pathogens.

F. Storage organs. All plants which have survived the evolutionary process in environments which include periods inimical to growth can only have survived as a result of the development of mechanisms whereby these periods are passed in a resistant state. Very severe conditions can normally only be survived in the form of seeds, but less severe conditions, which are not completely destructive, though not permitting growth to occur, can be survived in the form of buds in which the vulnerable growing point is protected and remains dormant during the inclement period and resumes growth and development when conditions improve. The bud may survive on the original plant and connected with its root system - as in the case of buds on woody perennial plants, or on cabbages or Brussels sprouts, discussed above - and draws upon the reserves in the plant when it resumes growth; or the bud may occur on a special storage organ which by structure or location is enabled to survive the inclement period although the rest of the original plant dies. The bud then draws upon the food reserves in the storage organ when growth is resumed.

All storage organs are capable of surviving the conditions under which they evolved as such, although in cultivation this capacity may have been impaired in the course of man's selection, not on the basis of potential for survival under natural conditions. but on the basis of yield and culinary quality. Potato tubers, for example, evolved as underground perennating organs on plants growing in the tropics, at altitudes up to about 4500m, in a climate varying from warm temperate to cool temperature with moderate rainfall and night frosts. We can expect the tubers to survive and remain viable in the environment provided by the soil, under such conditions, at a depth of, say, 10cm - a fairly constant temperature, low, though not below the freezing point; a high humidity, because of the lack of mass air movement, but not water-logged; an atmosphere in which, related to air (see inter alia Russell, 1950), neither oxygen depletion nor carbon dioxide accumulation occurs to a marked extent. We need not expect potato tubers to survive in an environment widely different from this. The function in the plant is of course, as stated, to survive and remain viable under the above conditions, not to remain palatable to man; and, while accepting the conditions enumerated as being suitable for survival, we must be prepared to modify them, if necessary, in the cause of palatability, remembering however that in so doing we may be detracting from storage life.

The buds on all storage organs eventually resume growth under suitable conditions, though not necessarily immediately after the conditions become favourable for growth - there may be a longer dormant period, some aspects of which could be regarded as a biochemical relic of the conditions under which the organ evolved. Renewed growth represents the natural termination of storage life. The storage hazards therefore are attack by pathogens; eventual growth; and any hazard, such as water loss, which we have introduced by storing a crop, not in the ground as in nature, but by methods which suit our convenience, our endeavour to retain palatability, and our frequent desire to store for longer than the natural life-span of the organ.

G. Fruits. If we seek an evolutionary survival value underlying the development of edible fruits it would seem to lie in providing a means whereby seeds are distributed at a distance from the competition of the parent, frequently perennial, plant. Rarely does an element of prolonged storage potential appear to form an essential part of the structure which has evolved, although a fleshy structure has an incidental advantage in respect, for example, to water loss, in that the surface/weight ratio is fairly low. To be effective as part of a method of dispersal, edible fruits need to become palatable to the distributor after the seeds are sufficiently ripe to remain viable after dispersal, but need remain palatable for only a comparatively brief time. There is no necessity, of course, that palatability to a distributor, frequently avian, should coincide with palatability to man, but in fact this is often the case.

On the basis of the foregoing, one might expect fruit in general to have no particular inbuilt storage advantages or disadvantages; nor any inbuilt tendency, or lack of tendency, to remain palatable for other than a brief period. Succulence can, however, be associated with susceptibility to damage with a resultant ready ingress of disease organisms and with the exudation of a nutrient solution. This causes fruit in general, and particularly soft fruit such as raspberries or strawberries, to be an admirable substrate for fungal growth. Also, although it was stated above that the surface/weight ratio of fruit is normally fairly low, which militates against evaporative loss, nevertheless the surface often permits quite a high rate of evaporation per unit area, and wilting can be a serious problem in fruit which it may be desired to keep for comparatively long periods. Pears provide a good example of this.

We can summarize the foregoing briefly as follows:

Material which is eaten immature and which is therefore in a state of rapid development when it is harvested (asparagus, broccoli, calabrese, cauliflower, button mushrooms, sprouting broccoli) will tend to continue that development, usually unacceptably, in store. Also the surface of such immature material is susceptible to rapid evaporative loss and the material is often fragile and liable to mechanical damage and bacterial and other rots.

Leafy material (kale, spinach, lettuce), because of its large surface/weight ratio, is subject above all to evaporative loss. It is not normally in a state of rapid change, and prevention of continued development is thus of minor importance. Like all plant material it is subject to rots.

Closely packed leafy material or buds (cabbage, Brussels sprout) evaporate mainly from the outer leaves and, particularly in the very dense forms such as winter-storing cabbage, evaporative loss has not the overriding importance it possesses in the case of ordinary leafy material. Because, however, buds have an inbuilt storage potential, as discussed above, we store them longer than ordinary leaves, and thus even a comparatively low rate of loss has a cumulative importance. Of potential importance, also, are loss of chlorophyll, undesirable flavour and odour changes, and bacterial and fungal rots. With very prolonged storage there is a tendency for the bud to grow.

Perennating organs, because of their natural potential, are the plant material which man has stored with greater or less success from time immemorial. Those with which we are mainly concerned, with the notable exception of the onion and its allies, are adapted to survive moderately low temperatures in the ground (beetroot, carrot, parsnip, potato, turnip). They are not adapted to survive desiccating conditions and are subject to evaporative loss. This, of course, is not nearly so rapid as in the case of leaves, but the time scale of storage is different - we store leaves for a few days, or at most a few weeks, but try to keep storage organs for several months. As in the case of buds, the cumulative loss can be serious, as can bacterial and fungal rotting. Sooner or later, if conditions permit - and often long before the end of the desired storage period - growth will occur.

The onion is an altogether different class to the other storage organs enumerated in that it evolved as an organ which could survive a hot dry period. It consists of leaf bases of which the outer ones are desiccated and provide protective layers which prevent rapid evaporation from the vulnerable swollen leaf bases which form the bulk of the organ. Dormancy is prolonged at high temperatures, unlike that of the other storage organs considered, though as in the case of other organs a sufficiently low temperature prevents growth. Rotting remains a hazard, particularly if the bulb has been exposed to moist conditions.

Fruits when ripe are often ephemeral and storage is potentially brief, the conditions being mainly adapted to prevention of development proceeding to the

senescent stage and avoiding fungal rots, which are of prime importance. It is, however, possible to harvest several fruits unripe (pears and tomatoes are typical examples) and thus add, to the normal storage life, a period during which the ripening processes occur. By suitable adjustment of the storage conditions, to slow down the biochemical changes, the period thus added may be a multiple of the natural storage potential of the ripe fruit. If this results in a fairly long storage period the cumulative evaporative loss may be serious.

Examples of the types of deterioration which terminate the useful storage life of a number of commodities are given in Table 1. It will be noticed that, in nearly every case, microbial attack features as a cause of loss, though not necessarily the major cause - in fact it has been omitted from two commodities listed only because, in practice, their useful storage life is nearly always terminated for other reasons. Both would be subject to rotting at a later stage. The pathogens responsible for the spoilage vary with the commodity and no attempt has been made to specify them in Table 1. The information given does, however, show that whatever methods are adopted for reducing storage losses, they must always be used in the knowledge that there is an ever-present possibility of microbial attack, and solving one problem could, not infrequently, give rise to another.

Table 1

Examples of the main types of deterioration terminating useful storage life (based on Burton, 1972, with additions)

Commodity	Storage terminated by
Apple	Senescence, microbial attack, wilting
Asparagus	Lignification, wilting at butt end, loss of flavour
Bean (runner)	Lignification, wilting, microbial attack
Beetroot (bunching)	Wilting
Beetroot (storing)	Wilting, regrowth, microbial attack
Blackberry	Microbial attack
Brussels sprout	Wilting, leaf senescence (yellowing), microbial attack, off-flavours and odours, discoloured butts
Carrot (storing)	Wilting, microbial attack, regrowth
Cabbage (winter-storing)	Microbial attack, wilting, leaf senescence, regrowth (bursting)
Cauliflower and Calabrese	Microbial attack, opening of florets, wilting, discoloured butts
Lettuce (cabbage)	Wilting, bruising, microbial attack, discoloured butts
Onion (bulb)	Microbial attack, growth
Pear	Senescence, wilting, microbial attack
Plum	Fruit collapse and microbial attack
Potato	Microbial attack, wilting, sprout growth
Raspberry	Microbial attack
Spinach	Wilting, microbial attack
Strawberry	Microbial attack
Tomato	Senescence, microbial attack

METHODS OF REDUCING STORAGE LOSSES

The three categories of storage loss will be considered separately.

Evaporative loss

Evaporation from any given commodity is directly proportional to the water vapour pressure deficit of its immediate environment. We have already considered in general terms the relative susceptibility of various commodities to evaporative loss; and some idea of the actual rates of loss involved. and the effects upon acceptability, can be derived from Tables 2 and 3. Nothing we can do will alter the relative ranking of the commodities as given in these Tables, unless we coat them with, for example, wax coatings. Apart from this the only method of reducing evaporative loss is to reduce the water vapour pressure deficit of the immediate environment and this is the first essential in the storage of most commodities - onion being the main exception. The acceptable maximum wvpd for any given storage time can be calculated from these Tables. For example, if we wish to store lettuce for 7 days (= 168 hours) and accept that 5% loss is the maximum permissible (Table 3), then the rate of loss must not exceed about 0.03% per hour. This means that the wvpd of the immediate environment must not exceed 10 Pa (Table 2). This entails in fact an almost saturated atmosphere - the water vapour pressures of air saturated at various temperatures are given in Table 4 and show that even at 0°C the degree of saturation would need to be 98.3% to provide such a low wvpd.

Table 2

Typical rates of water loss from various stored commodities (modified from Burton, 1972)

Commodity	Water loss (per cent $hr^{-1}Pa^{-1}$)+
Asparagus	1.5×10^{-3}
Beans (scarlet runner)	Related to size and maturity; e.g. 5×10^{-4} in c. 50g beans, 1.5×10^{-3} in c. 10g beans
Beetroot (bunching)	3×10^{-3} falling in the course of a few days to 8.5×10^{-4} as the leaves dry out
Beetroot (storing)	6.5×10^{-4}
Brussels sprouts	1×10^{-3} falling in the course of days to 8×10^{-4} as outer leaves dry out
Carrots (Nantes 20)	8×10^{-4}
Lettuce (cabbage, Hilda)	3×10^{-3}
Onions (bulb)	2×10^{-6}
Peppers (green)	2.5×10^{-4}
Potatoes (mature maincrop)	1×10^{-5}

+These figures multiplied by 100 give loss as per cent original weight per hour per mb water vpd. They are based on unpublished measurements by J.E. Robinson and K.M. Browne at the Food Research Institute, Norwich.

Table 3

Approximate maximum permissible water loss from various commodities (from Burton, 1972)

Commodity	per cent loss	Commodity	per cent loss
Asparagus	8	Carrots	8
Beans (runner)	5	Celery	6
Beetroot (bunching)	5	Lettuce	5
Beetroot (storing)	10	Potatoes	10
Brussels sprouts	6		

A low wvpd in the immediate environment of a commodity can be achieved in part by controlling the humidity of the store air, but this may need to be supplemented by reducing the volume of air in the environment - as by packaging the commodity in small units in, for example, polythene bags, perforated or unsealed to permit sufficient gaseous diffusion for respiration. The evaporation of the commodity into the restricted atmosphere of such a package rapidly raises the humidity to saturation - the small amount of evaporation needed to achieve this can readily be calculated, for any given volume of air in the package, from the figures in Table 4. For example, let us suppose we are storing lettuces, exposed to free air circulation, in a room at 2° and 90% RH. The wvpd in the room would be 70 Pa (Table 4) and the rate of loss over 0.2% per hour (Table 2). Although the evaporation could be thought to be humidifying the room and thus reducing evaporation, this would not be occurring in actual practice. The moisture in excess of that present at 90% RH - the equilibrium struck between a number of variables including the cooler area and temperature differential - would be deposited on the cooler as ice. The evaporation from the commodity would remain at 0.2% per hour and in 24 hours the lettuce would verge on the unacceptable. Now suppose we place an individual lettuce, weighing 500g, in a polythene bag, leaving a volume of air, in addition to the already saturated air between the leaves, of 3 litres (approximating to the figures obtained in practice). This would initially be at 90% RH and hence would contain 15mg of water vapour (Table 4). Evaporation of a further 1.6mg from the lettuce (= 0.003% of its weight) would bring the air practically to saturation and no further evaporation, other than that resulting from air leakage, would occur. Even if the air in the bag had been perfectly dry and at a temperature of 20°, evaporation of 51.4mg from the lettuce (= 0.01% of its weight) would have saturated the air and prevented further evaporation. Where this method can be applied, therefore, restriction of the volume of air in contact with the commodity, as by placing it in a polythene bag, represents a very efficient means of maintaining a high humidity in the immediate environment and thus reducing evaporation - far more efficient than attempting to hold a whole store at a high humidity, and in practical terms quite independent of temperature and of the humidity of the ambient air, as can be seen from the examples used above. In one of these, water loss was over 30 times that of the other, but the absolute amounts were so small that this 30-fold increase was negligible in terms of weight lost.

In the case of commodities which are very subject to water loss, therefore, consideration should always be given to packing in boxes or bags to reduce the volume of air in contact with them. The size of the package, its permeability to gaseous diffusion, and its stowage in the store room are conditioned by two things: the necessity for sufficient rapid gas exchange to allow of respiration without the accumulation of harmful amounts of CO_2 or harmful depletion of O_2; and the necessity for sufficiently rapid conduction of heat within and from the package to avoid both overheating and any appreciable temperature gradient within it.

Information, relevant to this, on the rates of respiration, and associated heat production, of a number of commodities is given in Table 5.

Table 4

The content and pressure of the water vapour in one cubic metre of saturated air at a total barometric pressure of a standard atmosphere ($= 1.01325 \times 10^5$ Pa)

Temperature °C	Weight of water vapour g	Volume of water vapour litres (= % by vol x 10)	Pressure of water vapour mb ($= 10^2$ Pa)	Weight of water vapour per mb pressure g
0	4.84	6.00	6.08	0.80
1	5.18	6.44	6.53	0.81
2	5.54	6.92	7.01	0.80
3	5.92	7.43	7.53	0.79
4	6.33	7.96	8.07	0.79
5	6.76	8.54	8.65	0.78
6	7.22	9.15	9.27	0.78
7	7.70	9.80	9.93	0.78
8	8.21	10.49	10.63	0.77
9	8.76	11.22	11.37	0.77
10	9.33	12.00	12.16	0.77
11	9.93	12.82	12.99	0.77
12	10.57	13.69	13.87	0.76
13	11.25	14.62	14.81	0.76
14	11.96	15.60	15.81	0.76
15	12.71	16.64	16.86	0.76
16	13.50	17.74	17.97	0.75
17	14.34	18.90	19.15	0.75
18	15.22	20.12	20.39	0.75
19	16.14	21.42	21.70	0.74
20	17.12	22.79	23.09	0.74
25	22.80	30.87	31.28	0.73
30	30.04	41.88	42.43	0.71

Rotting by pathogens

Moulds, yeasts and bacteria are all of importance in causing storage rots of vegetables and fruit, the relative importance varying according to the substrate provided by the particular commodity, its reaction to attack, the degree of infection, and the storage conditions.

The precise response of the host/pathogen complex to the temperature, humidity and composition of the storage atmosphere - which are the variables with which we are concerned here - differs considerably, depending on the hosts and pathogens

involved, but there are certain general principles which help in defining storage conditions which are likely to be either hazardous or reasonably safe.

All organisms have a minimum temperature below which they cannot grow. With increasing temperature above this, the rate of growth increases to an optimum and then, with continuing increase in temperature, declines sharply and finally stops. Broadly speaking, in the case of fungi, there is usually a difference of about 20°C between the minimum and optimum temperature and about 5°C between the optimum and the maximum at which growth stops (Tomkins, 1952).

Table 5

Approximate rates of carbon dioxide production of various commodities in air

/Note that these rates are those immediately following placing at the different temperatures and adjustment can subsequently occur; see text. The figures should be used only as a guide to the comparative rates of different commodities. Unpublished determinations by J.E. Robinson and K.M. Browne/

	CO_2 production mg $kg^{-1} hr^{-1}$		
Temperature °C:	0	10	20
Commodity			
Asparagus	27	58	120
Beans (runner)	20	35	90
Beetroot (salad bunching)	11	20	40
Cabbage (January King)	6	26	57
Cabbage (winter storing)	3	8	20
Calabrese	48	110	260
Carrots (storing)	11	20	34
Celery	7	12	33
Lettuce (summer cabbage)	18	27	85
Onions (bulb)	3	7	8
Potatoes (mature maincrop)	2	4	8
Spinach	20	50	130
Turnips (bunching)	15	30	52

The above figures for CO_2 production, multiplied by 2.5, give heat production in kilocalories per metric ton, and, multiplied by 10, give it in B.Th.U. per ton.

The minimum, optimum and maximum temperatures vary with the organism, and there are interactions with the other variables of the environment, but some growth of some organisms will occur down to the temperature at which the commodity freezes (ca. -1 to -2°C). There is thus no absolutely safe temperature, but rotting by most pathogens becomes very slow as the temperature is decreased below 5°C. The general principle with respect to temperature, therefore, is that the storage temperature should be as low as possible without causing injury to the commodity, and in many cases about 0°C is optimal. There are several exceptions, however, which are discussed below under the heading of changes in composition and metabolism. There are also exceptions deriving from the effect of temperature upon

the response of the host to attack. Phoma rot of potatoes is a case in point. Phoma solanicola is a comparatively weak wound parasite which only invades the tuber effectively under conditions which adversely affect the formation of wound cork. The result is that the optimum temperature range for Phoma rots to develop is 0-5°C (Malcolmson, 1958; Kranz, 1959), over which range wound cork formation is absent, or, at best, patchy, although the optimum for growth of the fungus is about 25°C. The optimum storage temperature for avoiding such rots in potato tubers is thus 10°C for about a week or two to allow wound healing followed by the lowest temperature which does not lead to undesirable changes or injury.

The growth of micro-organisms is more vigorous the moister the environment, and ceases when the relative humidity with which the growth medium is in equilibrium falls below a certain level - usually about 95% for bacteria and about 75% for fungi, though some of the latter cannot grow below about 90% RH - Botrytis cinerea for example. Tomkins (1952) measured the rate of spread of this fungus on previously dried films of malt agar. At 10°C spread was fairly vigorous at 100% RH and decreased linearly as humidity was reduced, there being no growth at 91% RH. At 20°C, spread at 100% RH was twice as rapid as at 10°C and again was reduced as humidity was reduced, although in this case it was still about a sixth of the maximum at 91% RH and probably needed about 89% RH to prevent growth.

Fruit and vegetables are in equilibrium at a relative humidity not far short of 100% and in this respect provide an optimum medium for the growth of pathogens, which, once they are established in the tissue of a commodity, are subject to no limitation resulting from environmental humidity. Organisms on the surface, however, are subject to the micro-climate at the surface, the relative humidity of which, at any given temperature, results from the dynamic equilibrium struck between the ambient water vapour pressure deficit relative to the commodity, the rate at which the commodity can lose water under a given deficit, and the local rate of air movement. A high ambient humidity, a commodity susceptible to water loss, and a low rate of air movement, are all conducive to a humid micro-climate; and hence to the surface growth of micro-organisms, which can be followed by invasion of wounds, stomata or lenticels, and the establishment of rots in the tissue. Storage in boxes or bags, recommended above for commodities which are very subject to water loss, thus provides humidity conditions which are ideal for the establishment of rots. It is therefore essential that if this method of storage is employed for such commodities, it should be at a temperature unfavourable for the growth of micro-organisms - that is, as near 0°C as possible without injuring the produce. At normal ambient temperature, losses by rotting could far outweigh any saving by the reduction of evaporative loss.

Apart from temperature and humidity the composition of the atmosphere can influence the growth of spoilage organisms, but it is usually only at oxygen tensions appreciably lower than 5% that even strictly aerobic organisms, such as most fungi, are in any way controlled - indeed Shaw (1969) concluded that there was probably no control by low oxygen, of Botrytis cinerea and Rhizopus nigricans on strawberries, unless the oxygen concentration was reduced below 1%, or preferably below 0.5%. Reduction of the oxygen concentration to a level sufficient to have any effect in controlling aerobic organisms could lead to the inhibition of the natural defence mechanism of the host and, in the case of commodities subject to bacterial rots, to rotting by anaerobes - as, for example, the rotting of potatoes by Clostridia (Lund and Wyatt, 1972).

Carbon dioxide can reduce fungal rotting, and this is one of the advantages to be derived from controlled atmosphere storage at the higher CO_2 levels. 50% CO_2 will, for instance, completely suppress fungal rotting of blackcurrants at 4.4°C (see e.g. Smith, 1957). In considering the effects of controlled atmosphere storage on rotting it must be remembered that a rot is the result of an interaction between pathogen and host. Storage conditions can affect the growth of the pathogen, but

they also affect the host; and reduced fungal rotting of, for instance, apples in controlled atmosphere storage, probably results in the main from its effect in delaying ripening and the accompanying increased susceptibility of the fruit. Shaw (1969) concluded that the reduction by high CO_2 (5% and 20%) of the growth of Botrytis cinerea and Rhizopus nigricans on strawberries resulted from its effect on the metabolism of the fruit rather than on the pathogen.

In general, in most commodities, temperature and humidity, particularly the former, rather than atmospheric composition, are the characteristics of the storage environment which can usefully be varied to control rotting by pathogens; but although some pathogens can be controlled by this means, the growth of others is only delayed, and there are also several instances of the host being less tolerant to low temperatures than is the parasite. In such cases, the use of microbial inhibitors may provide the only practical answer, as in controlling the rotting of oranges by Penicillium digitatum. This is an example of the development of an export industry having been practically dependent on the discovery (Tomkins, 1936) of an appropriate fungicide. The pioneer work of Tomkins (1936, 1937) in introducing diphenyl-impregnated wraps, soon to be followed by the commercial trials of Farkas (1938, 1939), is now often forgotten. Sulphur dioxide, extensively used for the control of Botrytis on grapes (Winkler and Jacob, 1925; Jacob, 1929) is an equally successful post-harvest microbial inhibitor which also has stood the test of time in commercial application. Recently we have seen the increasingly widespread use of benomyl (methyl-(1-butylcarbamoyl)-2-benzimidazole carbamate) following the work of Delp and Klöpping (1968). If microbial inhibitors are used to prevent or reduce the rotting of fruit and vegetables we must bear in mind not only the effects on the pathogen, but possible effects on the quality of the commodity and on the consumer. The desirable characteristics of the chemical employed are thus much more critical than are those of fungicides used on the non-edible parts of plants, and microbial inhibitors have been considered in general terms from this point of view by Ingram et al. (1964). The application and use of post-harvest fungicides have been reviewed by Eckert (1967).

Changes in composition and metabolism

Chemical changes are subject to influence by temperature and by other factors, such as the concentration of reactants and accumulation of resultants. Control of these gives us reasonable scope in influencing chemical reactions and simple chemical equilibria in a desired direction. Translated into the environment of the plant cell, however, though the potential scope for control exists, the dynamic equilibrium resulting from the many hundreds of interlinked reactions which are proceeding is so complex that we can rarely be certain of the effect of changing any variable. This can be illustrated by a consideration of the rate of respiration of a potato tuber. The simple concept is that, over the range of temperature within which the tuber is not adversely affected either by heat or by cold, lowering the temperature by 10°C will reduce the rate of respiration to between a third and a half. In fact, the immediate response to lowering the temperature from 20°C to 10°C may approximate quite closely to this. Within a few days, however, the very many reactions in the tuber, of which the observed oxygen uptake and carbon dioxide output are merely the overt nett result, will have struck a new series of dynamic equilibria. As a result of this the rates of uptake and output at 10°C are typically about three-quarters those at 20°C, rather than half. The effect of a further lowering of temperature, from 10°C to 0°C, is much more striking. Initially, as on the basis of the simple classical concept we should expect, uptake and output may be approximately halved; but the equilibria eventually re-established, over a period of a few weeks at 0°C, differ markedly from those which had existed at 10°C or at 20°C; and the uptake of O_2 and output of CO_2 at 0°C may not only exceed those at 10°C, but even those at 20°C (see e.g. Burton et al., 1955; Burton, 1974).

We can formulate the conclusions to be drawn from the foregoing in several ways, but I would suggest the following:-

1. As a first experimental approach to the problem of controlling undesirable metabolic change we can reasonably make use of general principles which have been derived in the main from observations on simple reactions and equilibria in vitro - for example, lowering the temperature decreases the velocity constant of a reaction; increasing the concentration of reactants increases the velocity at constant temperature; accumulation of the products of a reversible reaction progressively decreases the nett rate of change, eventually to zero in a state of dynamic equilibrium; and so on. This approach would often lead us to the correct qualitative conclusions as, for instance, to the effect upon respiration of lowering the temperature from 20° to 10° - and in most commodities, though not the potato, of lowering it still further; of decreasing the concentration of oxygen in the storage atmosphere; and of increasing that of carbon dioxide. We might sometimes obtain results other than we expected; but we could possibly be right more often than wrong, at least qualitatively if not quantitatively.

2. We must, however, realise that we are not dealing with a simple reaction, but with a whole host of integrated reactions, many reversible, with differing velocity constants and temperature coefficients, and catalysts which respond variously to environmental change. It is therefore unsafe to base our practice, or our advice, as opposed to our experiments, on extrapolated results - either from one temperature range to another (as in the example of the respiration of the potato tuber), or from one commodity to another, or even, in some cases, from one year to another, or one sample to another. It is necessary thoroughly to understand the relevant behaviour of the commodity before one can place limits of confidence on a prediction of its response to environmental change.

The first of the above conclusions indicates that the obvious method of delaying continued metabolic change in storage - such as opening of inflorescences, lignification, break of dormancy, and various forms of physiological deterioration which we can describe as senescence - may be to reduce the temperature. In practice, the method is almost invariably successful in giving a useful extension of storage life, provided that, in compliance with the second conclusion above, the vagaries of the commodity are known, with possible limitation of the minimum temperature which can be employed. In this connection we have already mentioned the respiratory behaviour of the potato tuber. Of more immediate practical concern is the fact that the potato accumulates sugar very markedly at low temperature, sufficiently to be unusable for some purposes. Thus although a temperature of about 2°, after initial wound healing, may be optimal for preventing sprout growth and storage rots - 0° is too low for some varieties, leading to internal discoloration and sometimes breakdown - it is too low a temperature for potatoes for crisping. Nevertheless, the fact remains that the temperature of storage should be as low as possible without causing too much sweetening for the market envisaged.

Similarly, if we are considering the short-term storage of unripe tomatoes, with a view to slow ripening in store to allow controlled marketing, then as a general principle the lower the temperature the slower the ripening. A temperature of 5°C or lower, however, leads to an uneven colour change, an uncharacteristic orange background colour, and a blotchy appearance. Again, the temperature should be the minimum possible without giving these undesirable symptoms - that is 7-8°C.

There are other well-known examples of intolerance to low temperatures. Bananas, for instance, should not be stored at temperatures below about 11-15°C, depending on variety, lower temperatures leading to a variety of symptoms, in part depending upon maturity - hard core in the fruit, a soft pulp in a green skin, a

a hard pulp in a yellow skin and so on. A temperature of 11-15°C is cool relative to tropical temperatures and thus represents cool storage for the banana; once again the temperature should be the minimum that does not give chilling injury.

Table 6 gives temperatures which have been recommended as optimal for the storage of various commodities. This list is confined to temperatures established by storage investigations at the Food Research Institute, Norwich, or its forerunners, the Ditton Laboratory, Kent and the Low Temperature Research Station, Cambridge.

Apart from reducing the temperature, other methods of lowering the rate of metabolism, suggested above, would be to decrease the concentration of relevant reactants or increase that of relevant products. Respiration is an index of the activity of stored material, and, on the assumption that the total potential activity is limited, an index of the rate at which a commodity is approaching the physiological end of its storage life. A reduction in the rate of respiration could thus be regarded as providing a means of extending storage life (see Kidd and West, 1927a); and application of the Law of Mass Action, to the simple concept of respiration as being the oxidation of a sugar to give carbon dioxide and water, was the rationale of the earliest experiments on controlled atmosphere storage, in which the carbon dioxide in the storage atmosphere was augmented and the oxygen was reduced. It was well understood by these early workers that the physiological basis, as just stated, was an over-simplification (Kidd et al., 1927), but they obtained in fact both a reduction in the rate of respiration and a correlated extension of storage life (Kidd and West, 1927b); though we now know that respiration and other processes occurring during ripening of fruit can be influenced separately (Dostal and Leopold, 1967; Frenkel et al., 1968; Rhodes, 1970). Controlled atmosphere storage has been widely applied, particularly to the storage of pome fruit, harvested unripe and ripened slowly under controlled conditions of temperature and atmospheric composition. In the early applications the increase in carbon dioxide and depletion of oxygen, achieved by storing the respiring fruit in a gas-tight chamber with a controlled air leak, were approximately equal, and the concentrations of each were around 10%. It was recognised at the very beginning, however, that separate control of the oxygen depletion and carbon dioxide increase would be preferable (Kidd et al., 1927) and this, with its possibility of combining, for example, low oxygen with low carbon dioxide, is now common practice in the case of commodities and varieties for which it has been found desirable. In Table 7 are given the combinations of temperature and atmosphere which have been recommended for a few commodities: for a much more extensive list, Stoll (1973) should be consulted.

The marked differences between the atmospheres which are optimal for different varieties of apple, as shown in Table 7, are a clear indication of the impossibility of recommending any storage conditions without adequate experiments on the commodity and variety concerned. The very high concentration of CO_2 recommended in Table 7 for blackcurrants is not, incidentally, an example of the use of a controlled atmosphere to reduce biochemical change, as in the other commodities listed, but an example of the use of high CO_2 to reduce fungal rotting.

CONCLUSION

The foregoing survey has attempted to give some indication of the ways in which the nature of a commodity can influence the forms of deterioration which are potentially important and of how these can influence the desirable characteristics of the storage environment. Many points of detail have been omitted; some of great importance, such as the production of ethylene by some commodities, and its effects, both beneficial, as in the controlled ripening of bananas, and detrimental, as in causing the wilting of the petals of cut flowers.

Table 6

Temperatures which have been recommended as optimal for the storage of commodities in air

Commodity		Temperature °C and authority
Apple (Blenheim Orange)		3.5 (K.W.)
Apple (Newton Wonder)		1 (K.W.)
Asparagus		0 (R.B.)
Beans (runner)		7 (R.B.)
Beetroot (Detroit Globe Improved)		4 (S.6)
Brussels sprouts		0 (R.B.)
Cabbage (winter storing)		0 (R.B.)
Carrots (Amsterdam Forcing)		2 (S.6)
Carrots (storing)		2 (R.B.)
Cauliflower		0 (R.B.)
Calabrese		0 (R.B.)
Celery		0 (R.B.)
Cucumber		7 (R.B.)
Gooseberry (Careless)		0 (S.6)
Lettuce		0 (R.B.)
Marrow		5 (R.B.)
Mushroom (button)		0 (S.5)
Onion		0 (R.B.)
Pear (Conference)		-1 (F.N.)
Potato (ware and canning)		4 (B.)
Potato (other processing)	short term	10 (B.)
	long term	7 (B.)
Raspberry		0 (R.B.)
Spinach		0 (R.B.)
Strawberry		0 (R.B.)
Tomato ($\frac{1}{4}$ ripe)		7-8 (R.B.)

Authorities: B. Burton (1972)

K.W. Kidd and West (1950)

R.B. Unpublished work by Robinson and Browne (Food Research Institute). In cases where the recommended temperature is 0°C, and temperature control in the store is doubtful, Robinson and Browne suggest 2°.

S.5 Smith (1965)

S.6 Smith (1966)

F.N. Fidler and North (1966)

Table 7

Conditions which have been recommended for the controlled atmosphere storage of various commodities

Commodity	Temperature °C	Atmosphere % CO_2	Atmosphere % O_2	Reference
Apple				
Bramley's Seedling (UK)	3-4	8-10	c.11-13*	Fidler & Mann (1972)
Cox's Orange Pippin (UK)				
(to Feb)	3.5-4.5	5	3	" " "
(beyond Feb)	3.5-4.5	1	1.8-2.5	" " "
Cox's Orange Pippin (Swiss)	4	1-2	3	Stoll (1972)
Golden Delicious (Swiss)	2.5	5	3	" "
or:	2.5	4	2	" "
Jonathan (UK)	4.5	6	3	Fidler & Mann (1972)
Jonathan (Swiss)	4	3-4	4-3	Stoll (1972)
King Edward VII (UK)	3.5-4	8-10	c.11-13*	Fidler & Mann (1972)
Pear				
Conference (UK)	0.5-1	5	5	Fidler & Mann (1972)
Conference (Swiss)	0	2	2	Stoll (1972)
Doyenne du Comice (UK)	0-0.5	5	5	Fidler & Mann (1972)
Doyenne du Comice (Swiss)	0	2	2	Stoll (1972)
William's Bon Chretien (UK)	0.5-1	6	15*	Fidler & Mann (1972)
William's Bon Chretien (Swiss)	0	2	2	Stoll (1972)
Blackcurrant	2-4	40-50	6-5	Stoll (1972)
Cauliflower (Swiss)	0	0-3	3-2	" "
Cucumber (Swiss)	14	5	5	" "
Cabbage (Swiss)				
Red	0	3	3	" "
Savoy	0	3	3	" "
White	0	0-3	3	" "

*No scrubber. CO_2 controlled. O_2 + CO_2 assumed to total 21%

So far as any rules general to all commodities can be deduced they are as follows:

1. The temperature should always be the lowest which gives no undesirable effects. In some commodities this can be as low as -1°C (Conference pears in air): in others as high as 15° (Lacatan bananas).

2. The humidity should be as high as possible without leading to microbial rotting. In the onion this can be as low as 70% RH. If, as in the case of leafy vegetables, a very high humidity, approaching 100% RH, is essential, then a low temperature (0-2°C), to discourage microbial growth, is also essential. High humidity without low temperature could lead to considerable rotting.

3. Controlled atmosphere storage (excluding, for example, additives such as ethylene, or the use of very high concentrations of CO_2 to reduce fungal rotting) is a means of delaying biochemical change. It is of practical use only for commodities in which this is potentially rapid and deleterious - mainly immature or unripe produce.

4. Careful storage reinforces the natural storage potential of a living commodity but does not change it. In very ephemeral produce we can think only in terms of days, or perhaps weeks, to help in controlling marketing. Only in the case of natural perennating organs can we think of storing for many months.

5. Inevitably, storage conditions which permit the survival of living plant material will also permit the survival of pathogens. The optimum conditions for host and pathogen may differ, but there will always be a range of overlap. Practical storage conditions should be adjusted to make this range as small as possible and to favour the host, but control of disease by this means can never be 100 per cent effective, and in some cases the use of microbial inhibitors is commercially essential.

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STRUCTURE/FUNGICIDAL ACTIVITY RELATIONSHIPS OF NEW ORGANIC SALTS OF SEVERAL ARYLSULPHONANILIDES

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Summary: Several 1-S-n-alkylisothiouronium salts of some p-toluenesulphonanilides proved to be considerably more fungitoxic than the sodium salts of the same sulphonamides, the bromide or p-toluenesulphonate of the same cation. Although the activity of each compound depended upon the anion and cation yet the activity of the 1-S-n-octylisothiouronium cation appeared to be independent of the nature of the anion. Such differences were very low in the case of the radial growth and very high in the poisoned food and hyphal growth techniques. Sclerotium cepivorum was the most sensitive of 4 fungi tested and the differences between its response to different compounds were either low or negligible. Several salts promoted fungal growth especially in the case of Rhizoctonia solani and Aspergillus niger.

INTRODUCTION

According to the "Principle of Covalent-Electrovalent Organic Molecule" (El-Nawawy and El-Kheshin, 1960) the biological activities of many organic anions may be altered in the presence of certain organic cations and vice versa (cf. El-Nawawy and Abdel-Moneim, 1968; El-Nawawy et al, 1972). In pursuing this study of structure/fungitoxicity relationships several 1-S-n-alkylisothiouronium salts of certain p-toluenesulphonamides, H Br, and p-toluenesulphonic acids were prepared and studied for their fungitoxic properties by three different techniques. Toluenesulphonamides were found to be somewhat active in the control of wheat rust and other diseases, but in general they have not shown activity comparable to many fungicides (cf. Gassner and Hassebrauk, 1936; Hassebrauk, 1938; Hart and Allison, 1939; Mitchel et al, 1950). In general they were not of recommended fungicidal properties (Goldworthy and Gertler, 1949; 1958)

METHOD AND MATERIALS

I-Chemicals:

Anions: (from) p-toluenensulphonanilide (TA) p-toluenesulphon-o-toluidide (TOT), p-toluenesulphon-m- chloroanilide (TMC), p-toluenesulphon-p-chloroanilide (TCP), p-toluenesulphon-2:4-dichloroanilide (T-2:4-C), p-toluenesulphon-2:5-dichloroanilide (T-2-5-C), bromide (Br), p-toluenesulphonate (T).

Cations: 1-S-n-Butyl-(B), 1-S-n-hexyl- (HX), 1-S-n- heptyl- (HP), 1-S-n-octylisothiouronium (OT) and Na.

The final salts were prepared by double decomposition between a saturated aqueous solution of the sodium salt of each acid, and the acetone solution of the 1-S-n-alkyl-isothiouronium bromide. The compounds were recrystallised from acetone. Yields, uncorrected melting points, nitrogen, sulphur and halogen contents are recorded in Table 1. The compounds were dissolved in water containing 5% ethanol to satisfy M/500 (a), M/1,000 (b), M/2,000 (c) and M/4,000 (d) in the final test medium.

II-Test in liquid mineral surcrose medium:

The spore suspension of Aspergillus niger and Fusarium oxysporum, was prepared and the test in the liquid mineral-sucrose medium buffered by sodium monohydrogen phosphate and citric acid was carried out as described by El-Nawawy and Moneim (1968). The number of spores was 100,000 spore/ml in the final test solution. Four replicates were made. The tubes were incubated at 27°- 28°. Observation was done every 12 hrs during the first few days, then every 24 hrs. The tubes which remain clear for twelve days were reinoculated and left under observation for another 18 days.

TABLE 1

Yields, melting points (°C), and elemental analysis for the 1-S-n-alkylisothiouronium salts

Compound	Yield (%)	M.P.(°C)	N (%)		S (%)		Cl (%)	
			C	F	C	F	C	F
TA,B	90	71-72	11.08	11.31	16.88	16.66	---	---
TOT, B	80	110	10.68	10.43	16.28	16.43	---	---
TMT,B	93	107-108	10.68	10.56	16.28	16.10	---	---
TPT,B	83	119-20	10.68	10.67	16.28	16.47	---	---
T-2:4-C,B	93	124	9.37	9.21	14.28	14.43	15.84	15.78
T-2:5-C,B	85	102	9.37	9.16	14.28	14.54	15.84	15.60
TOC,B	90	85	10.15	10.26	15.47	15.23	8.58	8.45
TMC,HX	94	84	9.51	9,36	14.49	14.64	8.04	8.06
TMC,HP	96	67	9.22	9.03	14.05	13.95	7.79	8.0
TMC,B	96	96	10.15	10.07	15.47	15.25	8.58	8.55
TMC,OT	90	78	8.94	9.20	13.63	13.43	7.56	7.52
TPC,B	92	103	10.15	10.0	15.47	15.12	8.58	8.47
T,HX	76	133	8.43	8.20	19.27	19.29	---	---
T,HP	50	97-98	8.09	8.00	18.49	18.27	---	---
T,OT	90	104-105	7.78	7.73	17.78	17.69	---	---

III-Dry Weight Test:

In 100-ml conical flasks, mineral-sucrose medium (4ml) buffer solution (2ml) and distilled water were mixed and autoclaved. The solution of the chemical was brought up

to a definite concentration, followed by the addition of the inoculum. In the case of the spore-forming fungi, 1 ml of the spore-suspension stock was used as the inoculum. In the case of Rhizoctonia solani and Sclerotium cepivorum, 6mm discs of the 7 days (for the first) and 10 days old on PDA (for the second) were used.

The total volume in each flask was 20ml. The flasks were incubated for 7 days at 27 - 28° in the case of F. oxysporum, A. niger, and R. solani, and for 15 days at 20° in the case of S. cepivorum. This was followed by autoclaving, filtration through pre-weighed dried sintered glass funnels, washed with distilled water (3X15 ml.), dried at 80° for three hours, then left under vacuum in a dessicator containing phosphorus pentoxide.

The fungistatic activity of each concentration was expressed as a percent retardation (R) in the growth of the fungus mat according to the following formulae (Topps and Wain, 1957).

Table 2

Time, in hours, required for the earliest perceptable growth of A. niger and F. oxysporum in the presence of several compounds in mineral sucrose medium (pH 7).

Compounds		Concentrations							
		M/500		M/1,000		M/2,000		M/4,000	
Anion	Cation	AS	F	AS	F	AS	F	AS	F
TA	Na	60	84	48	72	48	48	36	36
TA	B	(360)	(840)	(336)	(456)	144	(408)	120	240
TOT	Na	60	44	48	72	48	48	36	36
TOT	B	168	288	144	264	120	264	96	240
TMT	Na	60	84	48	72	48	48	36	36
TMT	B	192	264	168	216	144	168	120	120
TPT	Na	60	84	48	72	48	48	36	36
TPT	B	168	192	168	192	144	168	144	144
T-2:4-C	Na	60	60	48	48	48	48	36	36
T-2:4-C	B	240	(336)	240	288	216	240	192	168
T-2:5-C	Na	60	60	48	48	48	48	36	36
T-2:5-C	B	240	(432)	240	(432)	216	(408)	192	(408)
TOC	Na	60	72	48	60	48	48	36	36
TOC	B	216	264	216	216	168	192	168	144

Anion	Cation	M/500 AS	M/500 F	M/1,000 AS	M/1,000 F	M/2,000 AS	M/2,000 F	M/4,000 AS	M/4,000 F
TPC	Na	72	168	72	144	60	120	48	96
TPC	B	(408)	(456)	(456)	(456)	(360)	(504)	(360)	(504)
TMC	Na	60	72	48	60	48	48	36	36
TMC	B	192	264	168	240	144	240	120	216
TMC	HX	(408)	(384)	216	(360)	168	288	144	264
TMC	HP	(---)	(---)	(720)	(---)	288	(504)	264	(456)
TMC	OT	(---)	(---)	(---)	(---)	(---)	(504)	(408)	(408)
Br	B	60	72	48	60	48	48	36	48
Br	HX	96	96	72	72	60	60	48	60
Br	HP	(408)	(---)	(456)	(504)	96	72	60	60
Br	OT	(---)	(---)	(---)	(---)	(720)	(720)	(480)	120
T	Na	60	50	50	36	48	36	48	24
T	B	60	72	48	48	48	48	36	36
T	HX	96	96	72	72	60	60	48	36
T	HP	(---)	(---)	(456)	120	84	72	60	48
T	OT	(---)	(---)	(---)	(---)	(720)	(---)	(432)	(408)

AS = A. niger, F. oxysporum.

Values between brackets refer to the cases where reinoculation was carried out 12 days after the first inoculation.

(---) = No growth observed for 30 days in spite of reinoculation.

Table 3

Percent toxicity of M/2,000 of different compounds to the radial growth of the four fungi

Anion	Cation	pH	A. niger	F. oxysporum	R. solani	S. cepivorum
TMC	Na	5	20	18.9	17.4	64.6
		7	17.6	10.6	31.9	44.5
		8	28.0	19.5	41.1	63.3
TMC	HX	5	11.1	15.2	14.5	66.6
		7	(20.5)	15.2	12.2	69.0
		8	(9.3)	21.5	35.5	58.3
TMC	HP	5	14.5	23.9	21.2	64.0
		7	(17.6)	21.0	18.7	47.8
		8	(9.3)	22.0	43.5	100
TMC	OT	5	16.7	24.3	23.9	58.6
		7	(17.6)	11.0	15.2	66.7
		8	(9.3)	41.3	23.0	100
T	Na	5	11.1	(11.6)	0.0	66.6
		7	4.4	4.6	0.0	100
		8	6.6	44.0	1.7	100
T	HX	5	11.1	6.7	8.1	80.0
		7	4.4	40.6	89.7	83.0
		8	20.0	62.6	12.0	100
T	HP	5	11.1	13.9	9.2	81.3
		7	8.7	58.4	9.1	86.7
		8	60.0	67.6	16.3	100
T	OT	5	16.7	32.8	11.6	77.3
		7	100	64.6	12.0	100
		8	100	100	35.5	100

Values between brackets refer to activation of fungual growth.

Table 4

Percent toxicity of different compounds to the hyphal growth of the four fungi

Compound Anion	Cation	Concentration	A. niger	F. oxysporum	R. solani	S. cepivorum
TMC	Na	a	64.3	100	100	100
		b	68.7	50.7	(97.8)	100
		c	69.7	49.3	46.4	100
		d	57.7	32.3	(199.3)	100
TMC	HX	a	100	100	100	100
		b	68.7	58.9	(128.9)	100
		c	65.0	77.4	35.2	100
		d	58.8	63.9	(109.6)	100
TMC	HP	a	100	100	100	100
		b	100	100	100	100
		c	100	88.9	66.4	100
		d	100	69.5	(84.1)	100
TMC	OT	a	100	100	100	100
		b	100	100	100	100
		c	76.8	88.9	25.6	100
		d	61.1	64.3	(58.6)	100
T	Na	a	71.4	29.2	100	100
		b	63.0	5.9	48.9	62.8
		c	62.8	49.3	80.0	50.8
		d	54.9	41.9	(176.5)	51.5
T	HX	a	100	100	100	100
		b	76.3	68.9	100	100
		c	71.8	25.2	(16.8)	100
		d	59.9	(26.3)	(959.3)	100
T	HP	a	100	100	100	100
		b	100	100	100	100
		c	100	75.9	100	100
		d	91.4	(18.0)	72.4	100
T	OT	a	100	100	100	100
		b	100	100	100	100
		c	100	100	100	100
		d	100	69.2	100	100

a = M/500, b = M/1,000, c = M/2,000, d = M/4,000

Values between brackets refer to activation.

E = 100(Wc - Wt)/Wc where, Wc = dry weight of the fungal growth in the control and, Wt = dry weight of the fungal growth of the treated fungi.

The degree of activation (A) was calculated by the same formulae.

IV- Radial growth test:

A definite volume of the mineral-sucrose medium (4 ml, containing the required amount of agar) together with a calculated volume of distilled water were autoclaved in test tubes (1.5 x 18cm). The required volume of the buffer (2 ml) was autoclaved separately, then both solutions were mixed in the test tubes. The calculated volume of the fungicide was finally added, the contents of each tube medium, a disc inoculum (6mm in diameter) was placed in the centre of the Petri-dish. Three replicates were made for each treatment. In the case of A. niger a sterilised filter paper disc (6mm in diameter) was dipped in a stock solution of the spore suspension, dried and inserted with sterile forceps in the centre of the Petri-dish. Two vertical radii of the growth in each Petri-dish were measured every 24 hr. The percentage toxicity was calculated by the previously mentioned formulae of Topps and Wain (loc. cit.) as follows:

% Toxicity = 100(A - B)/A where A = diameter of untreated fungus, B = diameter of treated fungus. When the growth in any treatment filled the Petri-dish, the experiment was ended.

RESULTS

Test in liquid mineral-sucrose medium (Table 2): The compounds, at M/4,000 can be divided, according to their activities, into the following groups:

Weakly or non-toxic		moderately toxic		toxic		very toxic	
24 - 96 h		120 - 192 h		216-288 h		(312) - (-)	
As	F	As	F	As	F	As	F
All Na Salts							
		TA,B		TA,B			
TOT,B		TMT,B	TMT,B		TOT,B		
Br,B	Br,B	TPT,B	TPT,B				
Br,HX	Br,HX	T2:4-C,B	T2:4-C,B				T2:5-C,B
Br,HP	Br,HP	T-2:5-C,B					
		TOC,B	TOC,B			TPC,B	TPC,B
T,Na	T,Na	TMC,B			TMC,B		
T,HX	T,HX	TMC,HX			TMC,HX		
T,HX	T,HP			TMC,HP			TMC,HP
						TMC,OT	TMC,OT
			Br,OT			Br,OT	
						T,OT	T,OT

In general the following points can be recorded: (a) The sodium salts of TA, TOT, TMT, TPT, TOC, TMC, TPC, T-2:4-C, and T, as well as (Br, B) were weakly active. The B salts of all the sulphonamides were very active against A. niger and F. oxysporum (TPC,B) (TA, B) (T-2:5-C,B), (T-2:4-C,B) and (TOC,B) were the most active in descending order. F. oxysporum was, however, more susceptible than A. niger. (b) In the case of the HX, HP, and OT salts of TMC and Br the activity was in the following descending order with respect to the cation: OT, HP, HX, and in the following descending order with respect to the anion: TMC, T and Br. In general the activity of each molecule depended upon both parts of the molecule.

Radial growth experiment:

From Table 3 it is seen that: (a) S. cepivorum was the most sensitive to the test compounds. (b) The toxicity of T,Na to the other three fungi was very low, but it jumped considerably with T,OT especially at pH 7 pH 8. (c) The sulphonamide derivatives were not very active against three fungi; their isothiouronium derivatives stimulated the growth of A. niger at pH 7 and pH 8.

Hyphal weight experiment:

From Table 4 the following points can be recorded: (a) S. cepivorum was the most sensitive fungus. Its growth was stopped completely by all the concentrations of all the compounds except T,Na at the three lower concentrations. (b) The growth of R. solani was completely inhibited by the higher concentration of all the compounds but encouraged by certain lower concentrations of all the compounds except (T,HP) and (T,OT). (c) Growth promotion of F. oxysporum was rarely observed, but that of A. niger was never observed. (d) The compounds which gave 100% inhibition at all concentrations were: all except T,Na (S. cepivorum) T,OT (R. solani), TMC, HP, T,OT (A. niger) and none in the case of (F. oxysporum).

DISCUSSION

The penetrability of organic molecules through the biological membranes depends, in part, upon their physical properties especially the H/L ratio. This has been adjusted in the case of fatty solids, for example, through (a) the elongation of the aliphatic chain or (b) through the replacement of the hydrogen ion by an organic cation e.g. S-alkylisothiouronium (cf. El-Nawawy and Moneim, 1968). Once inside the cell, the two oppositely charged ions of the molecule might act independently, synergise or antagonise each other (El-Nawawy and El-Kheshin, 1960). In the poisoned food test all these effects could have happened. The fungicidal properties of the S-alkyl isothiouronium bromides increased with chain length from C_4 to C_8 . Simultaneously the H/L ratio was expected to decrease in the same direction. Assuming that the bromide, p-toluenesulphonate and sodium ions were of no fungicidal activity, it can be said that each of the C_4 and C_6 isothiouronium cations and the sulphonamide anions possessed very low fungicidal activity. This might be due to unsuitable H/L ratio. In other words the anion and the cation were not inherently non-fungicidal, but it was a matter of penetrability. The behaviour of the C_8 isothiouronium bromide comformed to this explanation. All the C_8 inorganic and organic salts were however of the same order of fungicidal activity. This might indicate that the H/L ratio of the cation on its own was quite suitable for penetration of fungal cells and killing the fungus. The higher H/L ratio of the C_7 cation was adjusted in the presence of the sulphonamide anion and this led to higher toxicity.

From the results of radial growth (RGT) and hyphal weight (HWT) tests, S. cepivorum proved to be very sensitive to most of the (T) and (TMC) salts. The isothiouronium p-toluenesulphonates were more toxic than the sodium salts at pH 5.

R. solani was very tolerent to the same compounds (RGT) but very susceptible to some of the compounds at the same concentration (HWT). In the latter test (T,HP) and (T,OT) gave 100% inhibition of the growth of R. solani with respect to F. oxysporum and A. niger the isothiouronium salts were more toxic than (T,Na) or (TMC,Na) (HWT). This difference existed in the case of (T) salts (RGT).

Promotion of fungal growth was observed with A. niger (RGT) and R. solani (HWT), but rarely found in the case F. oxysporum (RGT and HWT). This phenomenon was more frequent with (TMC) isothiouronium salts. The surface undisturbed growth on solid medium (RGT) or liquid (HWT) might be responsible for giving results different from those obtained in the case of poisoned food technique where the tubes were shaken once every 12 or 24 hr during the experiment.

CONCLUSION

1- Initial studies using 1-S-n-alkylisothiouronium salts of N-substituted p-toluene sulphonamides showed promising fungitoxic properties of the resulting compounds.

2- The sulphonamide and 1-S-n-alkylisothiouronium ions appeared to be inherently fungitoxic, but they had to enter the fungual cell. This was achieved by joining the two ions electrostatically.

3- Growth promoting of certain fungi occurred under the conditions of undisturbed surface growth of the fungus.

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ORGANIC SALTS OF TRICHLOROACETIC AND 2:2-DICHLOROPROPIONIC ACIDS AS EFFECTIVE FUNGICIDES.

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Summary The well-known anionic herbicides : trichloroacetic and 2:2-dichloropropionic acids, might also act as fungicides if applied in the form of their 1-S-n-heptyl-, 1-S-n-octyl-, and 2-S-n-octylisothiouronium salts.

INTRODUCTION

Studies of structure/fungicidal activity relationships were made according to the principle of "Covalent-electrovalent Organic Molecule" (El-Nawawy and El-Kheshin, 1960). Modifications of the activity of strongly active fungicidal anions were achieved according to this principle (El-Nawawy and Ashry, 1965; El-Nawawy and Khalifa, 1966; El-Nawawy et al, 1970). This paper describes the previously unknown fungicidal activities of the organic salts of two well known anionic herbicides: trichloroacetate and 2:2-dichloropropionate. The phytocidal properties of these salts proved to be much stronger than the phytocidal properties of the inorganic salts (El-Nawawy et al, 1972). Deeper penetration of the tissues and cell by these compounds, as well as their effect on the chromosomes had been reported (El-Sadek, 1972 a and b).

METHOD AND MATERIALS

I-Compounds:

Anions: Trichloroacetate (TCA), 2:2-dichloropropionate (DCP). Cations:

H, Na, Ca, S-alkylisothiouronium cations where alkyl = 1-S-methyl (Me THU), 1-S-ethyl (Et THU), 1-S-propyl (1-Pr THU), 2-S-propyl (2-Pr THU), 1-S-butyl (1-B THU), 2-S-n-butyl (2-B THU), 1-S-(2-methyl)-propyl (1-iso-B THU), 2-S-(2-methyl) - propyl (2-iso-BTHU), 1-S-n-amyl (1-ATHU), 2-S-n-amyl (2-ATHU), 1-S-(3-methyl)-n-butyl (1-iso-ATHU), 1-S-n-heptyl (HP THU), 1-S-n-octyl (1-OTHU), 2-S-n-octyl (2-OTHU),

The salts were prepared by double decomposition between saturated aqueous solutions of the sodium salt of each acid and the concentrated acetone solution of the S-alkylisothiouronium bromides. Yields, uncorrected melting points, solubility and elemental analysis are recorded in Table (1).

II- Solutions:

The compounds were dissolved in distilled water to give the required concentrations in the final test medium.

III- Fungicidal test:

Test fungi: Fusarium oxysporum var. vasinfectum, Alternaria tenuis, Aspergillus niger and Rhizoctonia solani.

VI- Procedures:

The test was done by the poisoned food and the radial growth techniques (El-Nawawy et al, 1973).

RESULTS

From the fungicidal test in the liquid mineral-sucrose medium : (Tables 2 and 3) the following points can be reported (a) At all concentrations, the free acids, their Na, Ca, C_1, C_2, C_3 and C_4 -isothiouronium salts were completely non-fungitoxic to A. niger, F. oxysporum and A. tenuis, (b) With respect to the C_5 isothiourium salts the (1-ATHU) derivative of each acid was the most active isomer at M/200; it was toxic to the three fungi; while at M/400 it was active against A. niger and A. tenuis; at further dilution the activity disappeared, (c) The (1-iso-ATHU) derivatives came in the next descending order, (d) The other C_5 isomers were non-active, (e) At M/200 and M/400 (HPTHU), (1-OTHU) and (2-OTHU) salts of both acids inhibited the growth of the three fungi for more than 25 days, (f) The same level of toxicity was obtained at M/800 of the four C_8 isothiouronium derivatives with respect to the three fungi, and (HPTHU, TCA) with respect to A. tenuis and F. oxysporum and (HPTHU,DCP) with respect to A. niger, (g) At M/1600 the compounds which gave complete growth inhibition for more than 25 days were the 1-OTHU salts of TCA and DCP (A. tenuis, and F. oxysporum) and 2-OTHU,TCA) (with respect to A. tenuis); the activity of the C_8 salts of DCP with respect to A. niger was greater than that of the analogous TCA salt.

Table 1

Yield, uncorrected melting point, solubility and elemental contents of the S-alkylisothiouronium salts

Compound	Yield (%)	M.P. (°C)	solubility in water at 25°C	Percent Cl		Percent N	
				C	F	C	F
Salts of TGA							
Me THU	86	157-158	1.88	30.472	30.50	12.010	11.80
Et THU	85	143	1.86	29.554	29.53	11.330	11.23
1-Pr THU	82	142-143	1.94	27.200	27.48	10.730	10.71
2-Pr THU	79	144	1.87	27.200	27.55	10.730	10.80
1-B THU	84	149-150	1.94	25.818	26.02	10.188	9.85
2-B THU	81	129-130	1.83	25.818	25.71	10.188	10.30
1-isoB THU	80	143-144	1.81	25.818	25.61	10.188	10.31
2-isoB THU	80	140-141	1.84	25.818	25.90	10.188	10.11
1-A THU	75	145	1.62	24.567	24.60	9.680	9.61
2-A THU	69	103	1.81	24.567	24.30	9.680	9.62
1-isoA THU	70	144-145	1.77	24.567	25.00	9.680	9.66
HP THU	68	137-138	0.66	23.588	23.35	8.830	9.88
1-O THU	65	142	0.25	21.450	21.23	8.459	8.36
2-O THU	66	107	0.55	21.450	21.14	8.459	8.64
Salts of DCP							
Me THU	85	172-173	2.85	42.011	42.40	11.040	10.54
Et THU	81	163-164	2.49	39.813	39.95	10.460	10.11
1-Pr THU	80	159	2.94	37.833	32.28	9.940	9.39
2-Pr THU	72	157-158	2.93	37.833	37.94	9.940	10.06
1-B THU	81	162-163	1.42	36.040	35.91	9.510	9.96
2-B THU	76	143-144	1.70	36.040	35.94	9.510	9.55
1-iso-BTHU	64	153-154	1.63	36.040	36.38	9.510	9.15
2-iso-BTHU	66	145	1.92	36.040	36.45	9.510	9.43
1-A THU	74	155-156	0.78	34.410	34.04	9.046	9.09
2-A THU	73	80- 81	0.89	34.410	34.21	9.046	9.06
1-iso-ATHU	77	178	0.76	34.410	34.27	9.046	9.19
HP THU	69	173	0.92	31.555	31.80	8.210	8.26
1-O THU	77	167-168	0.28	30.298	29.99	7.960	8.06
2-O THU	78	122-123	0.36	30.298	30.31	7.960	7.86

F = Found C = calculated.

Table 2

Time, in hours, required for the earliest perceptible fungual growth at 27° and pH 7 in liquid mineral-sucrose medium in the presence of trichloroacetatec acid and its salts (50,000 spore/ml)

cation		M/200			M/400			M/800			M/1600		
		A	F	AS	A	F	AS	A	F	AS	A	F	AS
H		48	24	24	48	24	24	48	24	24	48	24	24
Na		48	24	24	48	24	24	48	24	24	48	24	24
Ca		48	24	24	48	24	24	48	24	24	48	24	24
Me	THU	48	24	36	48	24	36	48	24	24	48	24	24
Et	THU	48	24	36	48	24	24	48	24	24	48	24	24
1-Pr	THU	48	24	36	48	24	24	48	24	24	48	24	24
2-Pr	THU	48	24	36	48	24	24	48	24	24	48	24	24
1-B	THU	48	24	36	48	24	24	48	24	24	48	24	24
2-B	THU	48	24	36	48	24	24	48	24	24	48	24	24
1-iso-B	THU	48	24	36	48	48	24	48	24	24	48	24	24
2-iso-B	THU	48	72	36	48	48	36	48	24	36	48	24	36
1-A	THU	144	96	84	72	24	60	48	24	36	48	24	36
2-A	THU	48	24	84	48	24	36	48	24	36	48	24	36
1-iso-A	THU	96	48	84	72	24	84	48	24	36	48	24	36
HP	THU	--	--	--	--	--	--	--	--	240	48	60	168
1-O	THU	--	--	--	--	--	--	--	--	---	--	--	168
2-O	THU	--	--	--	--	--	--	--	--	---	--	72	168

-- = No growth than 25 days.

AS = A. niger, F = F. oxysporum, A = A. tenuis.

Time required for earliest perceptible growth of the three untreated fungi were, 48, 24, 24 hours respectively.

Fromthe fungicidal test by the radial growth method (Table 4) the following points can be observed : (a) The free acids, their Na and Ca salts were inactive, (b) The C_7 and C_8 isothiouronium salts were obviously active; F. oxysporum was more susceptible than R. solani; (c) 100% inhibition of the growth of F. Oxysporum was obtained with the isothiouronium salts at M/600 and by (1-OTHU, DCP) at M/1200. Over 80% inhibition of the growth of some fungus was obtained by the isothiouronium salts of TCA and DCP at M/1200 and by HPTHU and 1-OTHU salts of the two acids at M/2400; M/4800 1-OTHU salts of TCA and DCP were still able to cause a toxicity of more than 75%.

Table 3

Time, in hours, required for the earliest perceptible fungual growth at 27° and pH 7, in liquid mineral-sucrose medium in the presence of 2:2-dichloropropionic acid and its salts (50,000 spore/ml)

cation		M/200			M/400			M/800			M/1600		
		A	F	AS	A	F	AS	A	F	AS	A	F	AS
H		48	24	24	48	24	24	48	24	24	48	24	24
Na		48	24	24	48	24	24	48	24	24	48	24	24
Ca		48	24	24	48	24	24	48	24	24	48	24	24
Me	THU	48	24	24	48	24	24	48	24	24	48	24	24
Et	THU	48	24	24	48	24	24	48	24	24	48	24	24
1-Pr	THU	48	24	24	48	24	24	48	24	24	48	24	24
2-Pr	THU	48	24	24	48	24	24	48	24	24	48	24	24
1-B	THU	48	24	36	48	24	24	48	24	24	48	24	24
2-B	THU	48	24	36	48	24	24	48	24	24	48	24	24
1-*iso*-B	THU	48	24	36	48	24	24	48	24	24	48	24	24
2-*iso*-B	THU	48	24	36	48	24	24	48	24	24	48	24	24
1-A	THU	144	96	108	96	24	72	48	24	36	48	24	24
2-A	THU	60	24	60	48	24	60	48	24	24	48	24	24
1-*iso*-A	THU	96	24	60	72	24	60	48	24	24	48	24	24
HP	THU	--	--	--	--	--	--	--	96	216	192	48	132
1-O	THU	--	--	--	--	--	--	--	--	--	--	--	456
2-O	THU	--	--	--	--	--	--	--	--	--	364	72	240

-- = No growth for more than 25 days.

AS = *A. niger*, F = *F. oxysporum*, A = *A. tenuis*.

Time required for earliest perceptible growth of the three untreated fungi were 48, 24, 24 hours respectively.

Table 4

Percent inhibition of radial growth of F. oxysporum (F) and R. solani (R) at 27° and pH 7 in the presence of trichloroacetic, 2:2-dichloropropionic acids and their salts.

Compound	Percent toxicity														
	M/600				M/1200				M/2400				M/4800		
	TCA		DCP		TCA		DCP		TCA		DCP		TCA		DCP
	F	R	F	R	F	R	F	R	F	R	F	R	F	R	F
H	0	25	0	0	0	25	0	0	0	21	0	0	0	21	0
Na	0	25	0	0	0	21	0	0	0	15.7	0	0	0	15.7	0
Ca	0	25	0	0	0	25	0	0	0	21	0	0	0	21	0
HPTHU	100	85	100	81	91.3	81.2	87.2	53.6	81.0	49.4	80.8	31.5	61.8	43.1	65.9
1-OTHU	100	83.2	100	73.7	92.3	70.5	100	57.9	87.0	55.7	87.2	41.5	76.1	40	76.6
2-OTHU	100	85.2	100	91.5	86.6	70.5	91.4	57.9	76.1	49.4	76.6	40	61.9	44.1	59.6

DISCUSSION

The study of the structure/fungicidal activity relationships of the Na, Ca and the S-alkylisothiouronium TCA and DCP salts proved that the inorganic and C_1- C_4 isothiouronium salts were completely non-fungicidal, certain C_5 isothiouronium salts were moderately active and the C_7 and C_8 isothiouronium salts were strongly active. The inherent fungitoxicity of the C_7 and C_8 S-alkylisothiouronium cation was proved by El-Nawawy et al, (1973). This was explained in terms of suitable H/L ratio required for penetration of biological membranes. The presence of organic cation causes a serious alteration in the water solubilities of the salt, and the H/L ratio; thus the penetrability will be affected and hence the toxicity is expected to change. This should have happened with the C_7 and C_8 isothiouronium salts of TCA and DCP. These were expected to penetrate biological membranes better than the inorganic salts of either the cations or the anions. Such increase in penetrability of biological membranes by these (organic-organic) salts had been proved by El-Sadek (1972 a and b). Hence the fungitoxic properties of the C_7 and C_8 isothiouronium salts of TCA and DCP might be due to a more suitable H/L ratio and hence to a better penetrability of these compounds to the biological membranes of the fungial cells. Consequently these compounds may act as fungicides and herbicides.

Conclusion

By working according to the principle of "Covalent-Electrovalent" organic molecule it was possible to obtain compounds which would act as fungicides and herbicides.

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THE NATURE OF THE DOSAGE-RESPONSE CURVE OF HOMOLOGOUS SERIES OF COVALENT-ELECTROVALENT ORGANIC FUNGICIDES

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Summary In fungicidal tests using five fungi the values of the slopes of DR curves of 1-S-n-alkylisothiouronium o-, m- and p-chlorophenylmercaptoacetates were not equal but were higher than those of the DR curves of the sodium salts of the three acids. The values of ED_{50} and ED_{95} of the isothiouronium salts were however lower than those of the sodium salts. All the values depended upon the length of the S-alkyl chain, the fungus, the anion, the percence of ethanol and the initial temperature to which the fungus in the medium was exposed. Fungal-growth-promotion was observed at low dilutions of certain compounds.

INTRODUCTION

The quantitative study of the structure/fungicidal activity relationships of twenty-one 1-S-n-alkylisothiouronium arylmercaptoacetates by a modified spore germination and dry weight tests was done according to the principle of "Covalent-Electrovalent Organic Molecule" (El-Nawawy and El-Kheshin, 1960). The effect of different initial temperatures and concentrations of ethanol on the toxicity of the compounds were also taken into consideration.

Horsfall (1957) mentioned that the slopes of the dosage response-curves of the members of one homologous series were equal because of similar toxicity mechanism. His studies were confined to compounds of covalent structure. In this work this point was tested with respect to the (organic - organic) molecules with one electrostatic bond.

METHOD AND MATERIALS

I-Chemical:

Anions: o-Chloro-, m-chloro-, and p-chlorophenylmercaptoacetates. Cations: Na (1), 1-S-methyl-(2), 1-S-ethyl-(3), 1-S-n-propyl-(4), 1-S-n-butyl-(5), 1-S-n-amyl-(6), 1-S-n-hepylisothiouronium-(7). The acids, the S-alkylisothiouronium cations, and the salts were prepared by conventional methods.

The sodium and 1-S-n-alkylisothiouronium salts were dissolved in sterilised distilled water. The final concentrations were M/600 (A), M/1200 (B), M/5000 (C), M/10000 (D) and M/100,000 (E). Ethanol was added at certain concentrations to ascertain its effect on the activity of the compounds.

II-Biological work:

(a) Test Organisms: Fusarium oxysporum var. vasinfectum, Alternaria tenuis, Botrytis fabae, Aspergillus niger, Gloeosporium musarum, Rhizoctonia solani.

(b) Culture Media: Alternaria tenuis and Botrytis fabae were grown on mineral-sucrose medium; the other fungi were grown in PDA medium. This medium (0.5%) was also used as a stimulant for the germination of the spores of A. niger. The mineral-sucrose medium was used in the dry-weight experiments.

(c) Spore Suspension: The cultures of the spore forming fungi were incubated at 25 °C for ten days, and new growth was rubbed with a transference loop in the presence of sterile distilled water. The suspension was transferred aseptically to a special sterilised germinating bottle mechanically shaken for ten minutes, filtered through a sterilised non-absorbent cotton and adjusted to 4 to 4.5 millions of spores /1 ml in the solution the final concentration in the test solution ranged from 200,000 to 250,000 spores per ml.

(d) Procedure: 1-Spore germination test: Shafer's method (1952) with slight modifications was adopted for this test. This was done as follows. A definite volume of an aqueous 5% ethanolic solution of the compound, one ml of spore suspension and distilled water, up to 20 ml, were mixed in a sterile bottle. One ml of this suspension was inserted in a clean sterile Petri dish (5 cm inner diameter) which was then inserted in a larger Petri dish (19.5 cm inner diameter) (used as a humidity chamber by adding water, 15 ml), then wrapped tightly by a sheet of polyethylene. The whole system was incubated at 25 ± 2 °C for 16-18 hours. Five replicates were made. Direct counting of germinated and non-germinated spores were done under the high power. Five hundred spores were counted for each concentration. The percentage of non-germinated spores was recorded, and the fungitoxic action was estimated according to the well known Abbott's formulae.

To study the effect of the initial temperature on the fungicidal activity of the compound, the mixture containing the spores and the fungicide was pipetted in a sterile tube which was dipped for ten minutes in a water-bath at 20, 30, 40 or 50 °C. The tube contents were transferred to a small germinating Petri dish treated and examined as mentioned above. Any modifications in the mode of germination or in the hyphal growth was recorded.

(e) Procedure 2: Dry weight test: In 100 ml conical flasks mineral-sucrose medium (15 ml), (monohydrogen sodium phosphate - citric acid) buffer (3 ml) and distilled water were autoclaved. The chemical and the inoculum were added. The inoculum was one ml of the previously mentioned spore suspension or a 4 mm disc of 7-day old fungal growth of R.solani on mineral-sucrose agar medium.

The flasks were kept under mechanical shaking for a period of 15 days at 25 °C. This was follwed by autoclaving, filtration through preweighed dried sintered glass funnels, washed with distilled water (3 x 15 ml), dried again by heating at 80 °C for three hours, then left under vacuum for 15 minutes in a dessicator containing phosphorus pentoxide.

The fungistatic activity of each concentration was expressed as a percentage retardation (R) in the growth of the fungus mat, according to the following formulae (Tonns and Wain, 1957)

$$R = 100(W_c - W_t)/W_c$$

Wc = dry weight of control mat, Wt = dry weight of the chemically treated fungi.

The degree of activation (A) was calculated by the same formulae.

III-Drawing of dosage-response curves:

This was done according to the method of Litchfield and Wilcoxon (1948).

RESULTS

Germinating spore test:

The results are recorded in Table 1 from which the following observations can be recorded: (a) The 1-S-n-alkylisothiouronium salts were, in most cases, obviously more fungitoxic than the sodium salts of the three chloromercaptoacetic acids. In a few cases the sodium salts were more active than some, but not all, of the isothiouronium salts of the same anion. (b) A significant increase in the values of the slopes and decrease in the values of ED_{50} were observed for most of the isothiouronium salts. Taking the values of ED_{50} and the slope as criteria for the relative activities of the compounds against different fungi, the compounds can be arranged according to the data of Table 1 as follows (Table 2). The isothiouronium salts are referred to by the length of their S-alkyl chain (C_1, C_2, C_3, C_4, C_5 and C_6). (c) From Table 2 it can be seen that the compounds possessing the highest ED_{50} values did not always possess the highest slope value. The compounds which possessed the highest value for both parameters were: the C_6 salts of the three anions (A. tenuis), the C_6 and C_4 salts of o-chloro-, and p-chloro-anions, the C_5 salt of the p-chloro-anions (B. fabae). (d) Table 2 shows clearly that the position of the Na salts was invariably lower than all or most of the S-alkylisothiouronium salts depending upon the anion, the fungus and length of the alkyl chain of the cation. (e) Promotion of spore germination, with respect to that of the untreated fungi was observed in the following cases at M/10,000.

Fungi	Salts of chlorophenylmercaptoacetates		
	o-chloro	m-chloro	p-chloro
F. oxysporum	C_2,C_5	C_1,C_2	———
A. tenuis	Na,C_5	———	C_1,C_3,C_4
B. fabae	Na	C_1,C_2,C_4,C_6	C_1
G. musarum	Na,C_1,C_2,C_3,C_4,C_5	Na,C_1,C_3,C_3,C_4,C_5,C_6	Na,C_1,C_2,C_3,C_4,C_5,C_6

(f) In a separate experiment, the effect of different non-seriously toxic concentrations of ethanol on the toxicity of several salts of o-chlorophenylmercaptoacetates was examined. The data in Table 3 indicate that the interaction gave no effect, increase in toxicity, or growth promotion. The increase in fungitoxicity was observed with the following compounds and fungi.

Percent ethanol	Salts									
	Na		3		6		7			
2%	BT	GL	—	—	—	—	—	—	—	—
5%	BT	GL	—	GL	—	AL	—	AL	—	—
10%	BT	GL	F	GL	F	—	GL	—	F	BT

Growth-promotion was observed in the case of G. musarum in the presence of 2% ethanol and the 1-S-n-hexylisothiouronium salt. (g) In a third experiment the effect of different initial temperatures on the fungitoxicity of certain derivatives of o-chlorophenylmercaptoacetate was examined. All the fungi could not tolerate an initial degree of 50 °C. Increase in toxicity was observed at 40 °C in the case of the sodium (B. fabae) and 1-S-n-amylisothiouronium salts (B. fabae) and G. musarum. This was judged by the noticeable decrease in the ED_{50} and increase in the slope of the dosage response curve (DRC). Another case of increase in toxicity of the 1-S-n-hexylisothiouronium salt at 30 °C was noticed (F. oxysporum). Growth promotion occurred with F. oxysporum (Na and 1-S-n-hexyl salts at 40 °C), A. tenuis (Na, and 1-S-n-amyl salts at 30 and 40 °C), and G. musarum (Na salt at 30 and 40 °C). Decrease in toxicity with increase of initial temperature was noticed with F. oxysporum (Na and 1-S-n-amyl salts), A. tenuis (the 1-S-n-hexyl salt), B. fabae (the three salts), and G. musarum (the 1-S-n-amyl and 1-S-n-hexyl salts).

Hyphal weight test:

The results are recorded in Table 5. The 1-S-n-amylisothiouronium salt was the least toxic to all fungi except G. musarum which was the most sensitive to the three compounds. The most toxic compound was the 1-S-n-hexylisothiouronium salt. Growth promotion was observed in several cases.

DISCUSSION

The 1-S-n-alkylisothiouronium chlorophenylmercaptoacetates were more fungitoxic than the corresponding sodium salts. This agreed with expectation according to the principle of Covalent-Electrovalent Organic Molecule (El-Nawawy and El-Kheshin, 1960). The slopes of the (DR) curve of the salts of the same anion with the same fungus were not equal. This did not agree with the idea expressed by Horsfall (1956) who mentioned that, with the same fungus, the compounds belonging to the same homologous series should possess the same toxic mechanism and hence the same slope for DR curves. This could be true with the completely covalent compounds or inorganic salts of an organic ion. Such a molecule acts at one site in the cell. On the other hand, an "organic-organic" molecule consisting of two organic ions joined by an electrostatic bond, where each ion is expected to act as an independent fungicide inside the cell, acts at two sites and hence possesses two fungitoxic mechanisms. Thus the slope of the dosage-response curve (DRC) for the covalent molecule might be a function of one toxicity mechanism, while the DRC slope of the "organic-organic" molecule might be a vector of two toxicity mechanisms. That the DRC slopes of the members of the homologous series of the "organic-organic" molecules were not equal is to be expected. The differences in H/L ratios of these members would affect their penetrabilities, and hence the concentration of each ion in its site of action. Besides, one of the two ions might be more toxic than the other in such a way that one of them would act at the lower concentrations more than the other ion and, as the concentration is increased, the weaker ion starts to contribute to the toxicity. The H/L ratios were the factor which controlled these concentrations inside the cell. These ratios were not equal for all the members of the homologous series and hence the vector of the two reaction mechanisms of the two ions would not be the same for all the members.

The growth-promotion of certain fungi by low concentrations of certain Na and S-alkylisothiouronium chlorophenylmercaptoacetates agreed with what was previously discovered by Fawcett et al, (1955) and El-Nawawy and Goma (1968) in the field of plant - growth regulators.

Organic solvents might increase the permeability of organic membranes (El-Nawawy et al, 1961). The rusults so obtained with ethanol in this research were irregular. In some cases it increased the toxicity and, in others it increased the growth-promotion. The increase in permeability of the fungial membranes might explain the increase in toxicity, while the growth-promotion might result from decrease in permeability, or interaction between ethanol and fungicide. Ethanol could be metabolised by certain fungi (Cochrane et al, 1963).

The exposure of the fungi to different intial temperatures did not lead to a certain temperatures/activity relationship. In a few cases increase in fungi-toxicity occurred. This might be due to increased permeability of the protoplas-matic membrane of the fungi.

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Table 1

The response of different fungi to salts of chlorophenylmercaptoacetic acid.

	ED* 50					ED* 95					slope				
	F	AS	BT	GL	AL	F	AS	BT	GL	AL	F	AS	BT	GL	AL
o- Chlorophenylmercaptoacetic acid															
1-	1	1/600	1/24	1/590	1/26	1	1	1	1/6	1	0.35	0.40	6.7	0.9	0.60
2-	1/780	1/910	1/56	1/670	1	1/86	1	1	1/9.6	1	1.55	0.40	0.7	1.1	0.30
3-	1/770	1/7500	1/800	1/700	1	1/87	1/11	1/2	1/10	1	1.80	0.5	0.7	1.1	0.40
4-	1/790	1/8500	1/690	1/880	1	1/44	1/680	1/30	1/9.7	1	1.30	1.30	1.1	1.1	0.40
5-	1/400	1/8500	1/930	1/910	1	1/3	1/88	1/70	1/38	1	1.10	0.70	1.2	1.1	0.70
6-	1/850	1/6500	1	1/500	1/5.4	1/92	1/300	1	1/24		1.92	1.40	0.5	1.4	0.80
7-	1/5800	1/50000	1/992	1/910	1/5000	1/92	1/920	1/80	1/500	1/600	1.07	0.07	1.2	3.4	1.80
m- Chlorophenylmercaptoacetic acid															
1-	1/10	1/400	1/8.5	1/95	1/65	1	1	1	1	1	1.0	0.50	0.7	0.6	1.20
2-	1/810	1/900	1/44	1/440	1/9.4	1/70	1/8	1	1/8	1	1.44	0.70	0.8	1.1	0.62
3-	1/880	1/950	1/75	1/690	1/40	1/63	1/9.9	1	1/9.2	1	1.20	0.90	0.8	1.0	0.97
4-	1/4000	1/5800	1/94	1/70	1/77	1/650	1/90	1/6	1/9.5	1	2.08	1.02	1.1	1.0	0.80
5-	1/4800	1/6400	1/200	1/840	1/97	1/750	1/91	1/9.3	1/8.5	1	1.25	1.08	1.3	0.9	0.84
6-	1/8300	1/6000	1/790	1/440	1/100	1/820	1/97	1/80	1/9.3	1/4	1.67	1.18	1.7	1.1	1.01
7-	1/6500	1/35000	1/700	1/930	1/6000	1/700	1/700	1/76	1/840	1/700	1.80	1.12	1.7	2.6	1.84
p- Chlorophenylmercaptoacetic acid															
1-	1/30	1/750	1/99.8	1/100	1/10	1	1	1	1	1	0.90	0.40	1.0	0.4	0.70
2-	1/770	1/940	1/98	1/770	1/9.5	1/9.5	1	1/7.5	1/7	1	1.00	0.50	1.2	0.99	0.62
3-	1/760	1/960	1/880	1/840	1/8.5	1/30	1/100	1/8.8	1/9	1	1.18	0.80	1.2	0.99	0.72
4-	1/5200	1/8800	1/810	1/930	1/8.5	1/730	1/600	1/53	1/30	1	2.60	1.20	1.1	1.0	0.63
5-	1/6200	1/9000	1/960	1/980	1/10	1/850	1/610	1/85	1/91	1	2.28	1.23	1.2	1.4	0.60
6-	1/8000	1/7500	1/1000	1/600	1/8.6	1/820	1/500	1/93	1/9	1/7	1.70	1.32	1.2	1.2	1.20
7-	1/6800	1/60200	1/4900	1/950	1/300	1/750	1/998	1/98	1/300	1/9.6	1.80	1.20	1.7	3.10	1.30

F = F. oxysporum, AS = A. niger, BT = B. fabae, GL = G. musarum, AL = A. tenuis.

* Values are in molar concentrations.

Table 2

Comparison between activities of salts of the three chlorophenylmercaptoacetates

		o-Chloro-							m-Chloro-							p-Chloro-						
F	I	C_6	C_5	C_1=	C_2=	C_3	C_4	Na	C_5	C_6	C_4=	C_3	C_2=	C_1	Na	C_5	C_6	C_4	C_3	C_2=	C_1	Na
	II	C_5	C_2	C_1	C_3	C_4	C_6	Na	C_3	C_6	C_5	C_1	C_4=	C_2	Na	C_3	C_4	C_6	C_5	C_2	C_1	
AS	I	C_6	C_4=	C_3	C_2	C_5	C_1	Na	C_6	C_4=	C_5	C_3	C_2=	C_1	Na	C_6	C_4	C_3	C_5	C_2=	C_1	Na
	II	C_5	C_3	C_4=	C_6	C_2	Na	= C_1	C_5	C_6	C_4	C_3	C_2	C_1	Na	C_5	C_4	C_6=	C_3	C_2	C_1	Na
B	I	C_6	C_4	C_2	C_3	C_1	Na	C_5	C_5	C_6	C_4	C_3	C_2	C_1	Na	C_6	C_5	C_4	C_2=	C_3	C_1=	Na
	II	C_6=	C_4	C_3	C_1=	C_2=	Na	C_5	C_6=	C_5	C_4	C_3	C_2=	C_1	Na	C_6	C_5=	C_4=	C_2=	C_1	C_3	Na
GL	I	C_6=	C_4	C_3	C_2	C_1	Na	C_5	C_6	C_4	C_2	C_5=	C_1	Na	C_3	C_4=	C_6=	C_3	C_2	C_1	C_5	Na
	II	C_6	C_5	C_4=	C_3=	C_2=	C_1	Na	C_6	C_5=	C_1	C_2=	C_3	C_4	Na	C_6	C_4	C_5	C_3=	C_2=	C_1	Na
AL	I	C_6	Na	C_5	C_4=	C_3=	C_2=	C_1	C_6	C_5=	C_4	C_3	Na	C_2	C_1	C_6	C_4=	C_1=	C_2=	C_3=	C_5=	Na
		C_6	C_5	C_4	Na	C_3=	C_2	C_1	C_6	Na	C_5	C_2	C_4	C_3	C_1	C_6	C_5	C_2	Na	C_3	C_1	C_4

I = ED_{50} .

II = Slope .

Table 3

The response of different fungi to salts of o-chlorophenylmercaptoacetates in the presence of ethanol Compounds

	EtOH(%)	ED_{50}*				ED_{95}*				Slope			
		F	AL	BT	GL	F	AL	BT	GL	F	AL	BT	GL
I	0	10	1/87	1/10	___	10	1/7.2	10	___	0.12	1.4	0.4	___
	0	10	1/75	1/80	1/9.7	10	1	10	8	0.33	0.9	0.34	0.97
	5	10	1/26	1/24	1/590	10	10	1	1/6	0.4	0.6	0.70	0.9
	10	1/1.7	1	1/90	$1X10^{-7}$	10	10	10	1/9700	0.35	0.2	0.23	0.20
3	0	1/900	1/530	1/600	1/390	1/15	10	1/5	1/5	1.4	0.12	0.80	1.2
	2	1/930	1/97	1/730	1/440	1/98	3	1/5	1/8.8	1.6	0.6	0.75	1.2
	5	1/770	10	1/800	1/700	1/80	10	1/2	1/10	1.3	0.4	0.70	1.1
	10	$1X10^{-7}$	1	1/780	$1X10^{-6}$	10	10	1/6	$1X10^{-4}$	0.4	0.6	0.80	0.2
6	0	1/940	1	10	1/420	1/77	10	10	1/8.7	1.0	0.4	0.4	1.2
	2	1/860	1/9.6	10	1/600	1/88	10	10	1/10	1.6	0.54	0.6	1.3
	5	1/850	1/9.4	1	1/500	1/90	7	1	1/24	1.8	0.8	0.5	1.4
	10	10	10	10	1/2400	10	10	10	1/700	0.3	0.3	0.14	2.1
7	0	1/9500	1/820	1/9	1/870	1/940	1/70	10	1/440	1.4	1.6	0.5	3.3
	2	1/9500	1/900	1/6	___	1/860	1/84	10	___	1.8	1.8	0.3	___
	5	1/5800	1/5000	1/992	1/910	1/90	1/600	1/80	1/500	1.06	1.8	1.2	3.4
	10	1/50,000	1/680	1/9200	$1X10^{-7}$	1/960	4	1/93	$1X10^{-7}$	1.50	0.6	0.8	0.05

F = F. oxysporum, AL = A. tenuis, BT = B. fabae, GL = G. musarum.

* values are in molar concentration

___ = Growth promotion.

Table 4

The response of different fungi to salts of o-chlorophenylmercaptoacetates after exposure to certain initial temperatures

Compound	T	ED_{50}				ED_{95}				Slope			
		F	AL	BT	GL	F	AL	BT	GL	F	AL	BT	GL
1	20	1/40	10	1/60	1/70	10	10	10	5	1.3	0.24	0.15	0.8
	30	1/36	--	1/45	----	1/7.677	--	10	--	1.2	----	0.60	---
	40	----	--	1/400	----	--	--	1/9.9	--	---	----	1.50	---
6	20	1/890	1/70	1/600	1/440	1/92	1/708	1/20	1/8	1.85	1.1	1.7	1.01
	30	1/220	--	1/85	1/300	1/28	--	1/9.9	1/6	1.70	---	2.3	0.90
	40	1/500	--	1/870	1/99	1/92	--	1/480	1/6	1.90	---	3.4	1.0
7	20	1/6000	1/1200	1/8000	1/930	1/95	1/5000	1/7	1/200	1.0	1.7	0.6	2.30
	30	1/8500	1/360	1/4800	1/940	1/910	1/50	1/9.6	1/750	1.9	1.9	1.3	2.20
	40	----	1/890	1/960	1/3500	--	1/650	1/4	1/750	---	2.2	0.6	1.94

Table 5

Toxicity of different salts of o-chlorophenylmercaptoacetates to the hyphal growth of different fungi

Compounds		Percent toxicity F	AL	BT	GL	RS
Na	A	21	48.2	87.5	91.1	45.5
	B	35.4	39.2	50	90.2	(10.1)
	C	53.5	41.8	50	89.3	(20.6)
	D	----	15.2	12.5	87.4	(27.6)
	E	----	11.8	12.5	86.7	(3.5)
6	A	23	----	50	88.5	0.54
	B	(77.6)	----	25	89.1	(0.5)
	C	(7.6)	----	--	85.9	(22.4)
	D	(12.3)	----	--	85.6	(30.2)
	E	(33.4)	----	--	85.1	(30.0)
7	A	96.5	97.7	75	91.3	85.1
	B	84.5	84.7	62.5	88.7	16.7
	C	37.5	52.1	50	86.8	25.3
	D	----	30.5	25	90.2	(7.4)
	E	(11.5)	24.3	(12.5)	80.9	6.9

--- = No toxicity

( ) = % of growth promotion

NOTES

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Closing Remarks

by

D. J. Higgons

Mr. Chairman, Ladies and Gentlemen,

On behalf of your President, Sir Emrys Jones, who unfortunately has been called back urgently to Cirencester, I have been asked, as Chairman of the Programme Committee, to sum up the objectives and achievements of this, the 7th British Insecticide and Fungicide Conference.

These annual conferences are now, as has been reiterated by many of our eminent speakers, including the President himself, an International event of outstanding importance to our Agrochemical industry. Their evolution has been carefully guided by the operating sub-committees to reflect the principle objectives of the council itself which are:-

1. The promotion of informal contact between all members of the agrochemical industry, working in both the public and private sectors.

2. The provision of a multi-disciplinary platform for the rapid dissimination of scientific information throughout the industry.

The first of these two objectives is achieved by the generous hospitality provided by all the international companies supporting the Conference and by the social events organised by the Council itself.

The second is of course, the main function of the Programme Committee of which I have the honour to be Chairman.

When the first advisory committee took place in October 1972 to decide the main themes of this current conference, the decision was made to stand back somewhat from the specialised disciplines of our science to see how they fit into the farming scene as a whole, and we have been particularly fortunate in having had the honour of listening to Sir Henry Plumb's masterful survey in the First Bawden Memorial Lecture, which must have clarified in many of our minds, the place where our own individual activities fit to the whole.

From the scientific standpoint it is almost invidious to pick out any particular aspect, as the overall standard of all contributions and sessions has been so high. However, there are a number of very clear lessons which stand out.

In Sessions 9 and 10 this morning, the continued innovative activities of the industry, particularly in the insecticide field, are clearly indicated by the number of new compounds discussed and this must auger well for the future.

The various sessions on Cereal pathology, whether foliar or seedborne, reveal however the increasing importance of fungicides in this crop for the maintenance of our crop yields. With the capacity of cereal mildew alone to reduce barley yields by 40% and the appearance already of strains tolerant to some of the newer fungicides, we must view with concern the decreasing interest of many large industrial companies in fungicides for the arable sector due to the static nature of the total market potential for fungicides at an international level.

Surely here is an area for total co-operative research, if cereal yields are to be maintained and increased. It is also very clear that the problems of effective control of soil pests and diseases must increasingly occupy our attention if the theoretical yield capability of our soils is to be attained. Here there are signs of success although the integration of the use of individual compounds into spray programmes utilisable by farmers in practise has yet to be worked out.

The Programme Committee has also tried to bring in a number of fundamental sessions of which the popularity of the sessions on "Uptake and Translocation" was particularly heartening. The whole issue of Xylem versus Phloem transport must be of paramount importance to the ultimate breakthrough into the truly systemic fungicide.

In conclusion I would like to acknowledge our debt to the members of the Programme Committee without whose tireless effort this conference would not have been possible, as well as the contributions of all our Invited Speakers and Session Chairmen.

It would be invidious to pick out any particular names as all have contributed so ably. However, I personally would like to express my public thanks to the two Vice-Chairmen of the Programme Committee - Mr. Gair and Mr. Lester for their continued support and counsel.

Thank you very much indeed Ladies and Gentlemen, and I now declare the conference formally closed.

BRITISH CROP PROTECTION COUNCIL PUBLICATIONS

The following publications of the British Crop Protection Council can be obtained by post from Mr. A. W. Billitt, Clacks Farm, Boreley, Ombersley, Droitwich, Worcester.

Conference Proceedings

4th British Insecticide and Fungicide Conference, 1967
Vols. 1 and 2 .. .. £4.50

6th British Insecticide and Fungicide Conference, 1971
Vols. 1, 2 and 3 .. .. £7.50

7th British Insecticide and Fungicide Conference, 1973
Vols. 1, 2 and 3 .. .. £8.00

9th British Weed Control Conference, 1968
Vols. 1, 2 and 3 .. .. £7.50

11th British Weed Control Conference, 1972
Vols. 1, 2 and 3 .. .. £8.00

Monograph Series

No.1 - 1st Review of Insecticide and Fungicide Usage, 1970 .. 75p

No.2 - Symposium on the Application of Pesticides, 1970 .. .. £1.50

No.4 - 8th Review of Herbicide Usage, 1971 .. .. 75p

No.5 - 2nd Review of Insecticide and Fungicide Usage (Cereals), 1972 .. .. 75p

No.6 - 9th Review of Herbicide Usage, 1972 .. .. 75p

No.7 - 2nd Symposium on Herbicides in British Fruit Growing, 1973 .. .. £1.50

No.8 - 3rd Review of Insecticide and Fungicide Usage (Fruit and Hops), 1973 .. .. 75p