SOME NEW FINDINGS ON THE MODE OF ACTION AND THE METABOLISM OF TRIAZINE HERBICIDES

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Summary A review on the mode of action of the triazine herbicides and some detail information related to the metabolism of Prometryne are given.

INTRODUCTION

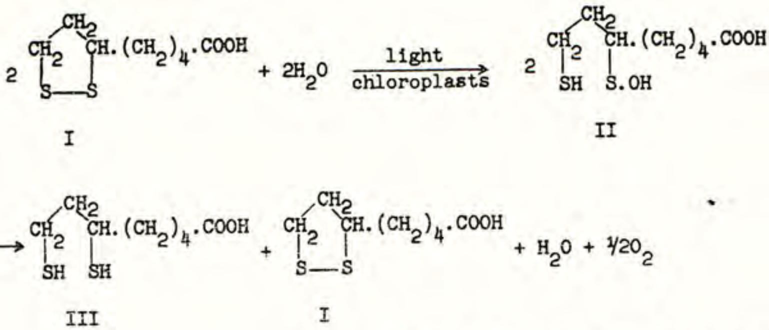
There are many questions to be answered as soon as a herbicidally active compound or a group of compounds approaches or enters the stage of becoming a commercial product: spectrum of activity with respect to weeds, safety-margin towards crop-plants, carry-over in the soil, residues and metabolites in crops, full toxicological evaluation, and many more. In the range of such questions, that of the mode of action plays a Cinderella role to some extent. The creating firms are busy with the burden of carrying out the experimentation for knowledge to meet the most urgent practical needs. Also the majority of the many experimental stations, who contribute in a most appreciable way to the practical evaluation of advanced research compounds, are, of course, rather inclined to check the usefulness under the conditions of their problems and their environment. But there are, fortunately, an increasing number of Experimental Station and University workers who devote their enthusiasm to the secrets related to the mode of action, and they merit the acknowledgement of all interested in the field. But you do not need me to tell you that the complexity of an organism like a plant and the different behaviour from plant to plant make the task of obtaining the detailed picture one of the most difficult and laborious ones. Therefore, although the review on related studies made on the triazines gives some very interesting findings, our knowledge is far from being complete and final.

MODE OF ACTION

Triazines penetrate plants through the cuticle and through the roots. In the case of some chlorodiaminotriazines with low lipid solubility, like Simazine and Propazine, phytotoxicity due to uptake by the leaves seems to be negligible under practical application conditions; but Crafts (1961) was able to show in laboratory experiments penetration and transport in acropetal direction in Simazine-treated bean leaves. Root uptake is very quick. Van der Zweep (1961) observed a change in the plant physiology even half an hour after immersion in a solution containing Simazine. Upward movement occurs in the xylem. Roots do not seem to be susceptible; their function remains for a long time intact and the speed of uptake is not reduced by root-inherent factors. As the herbicide is transported in the transpiration stream it was to have been expected, and Sheets (1959, 1961) confirmed, that higher amounts are taken up at higher temperatures, and more at lower than at higher relative humidity. As the speed of uptake varies from plant species to plant species, it can be considered as one of the factors determining sensitivity or resistance of a plant. In addition to the climatic factors, the triazine itself has a strong influence on the transpiration rate; Smith and Buchholtz (1962) at the University of Wisconsin and Davis and co-workers (1963; see Wills, G.D. et al.) at Auburn/Alabama observed a drastic reduction of the transpiration of maize, soya-beans and cotton. A close examination showed that this reduction was a consequence of the closure of the leaf stomata. It seems likely that biochemical changes in the guard cells are responsible for the closure. As will be discussed later, triazines influence the whole carbohydrate status of the plants, and Smith (1963), therefore, thinks that the closure is caused by the blocking of photosynthesis in the guard cells and the subsequent increase of CO_2. The question arises as to whether direct changes in the density of the cell sap and consequently in the turgor are responsible. It should be mentioned, however, that a team from Rothamsted Experimental Station (Waggoner, P.E. et al., 1964) and Zelitch (1964) from Connecticut described that stomatal closure induced by a group of other chemicals, alkenylsuccinic acids, was due to an increase of the permeability of the membranes, leading again to a decrease of turgor in the guard cells. So this possibility has to be kept in mind, too. It is worthwile noting, that the transpiration of the highly resistant maize is reduced also, but to a lesser percentage and is not followed by a collapse of the plant. The uptake of ions like the phosphate ion does not seem to be influenced, at least not in the early stages of triazine interference (Yeligar, M.B., 1963).

As already mentioned it has been well established that triazines interfere with the carbohydrate balance of the plants. Gast (1958) from our laboratories was the first to observe the depletion of starch in plants treated with triazines; he was able to counteract the action by floating isolated leaves in nutrition solutions containing saccharose. In such a way it became evident that triazines do not interfere with the build up of starch and further polysaccharides from monosaccharides, but with the building up of the monosaccharides themselves. These findings were confirmed by Moreland and Hilton from USDA and North Carolina Agricultural Station (1958). From this picture and from evidence known from earlier studies in the series of ureas, it was quite obvious that triazines could interfere with the Hill reaction. Exer (1958), from our laboratories, the Moreland-group (Moreland, D.E. et al., 1959) and Good (1961) from London, Ontario showed that this is in fact the case. Triazines figure among the most active Hill-inhibitors known. Their 50 % inhibition values, when determined on chloroplasts of spinach and Janus green used as a hydrogen acceptor, are at $7 \cdot 10^{-7}$ molare. This value can vary within certain limits according to the technique applied. Thus, the point of attack of triazines seems to be closely related to the photochemical reduction of DPN (diphosphopyridine nucleotide). The blocking of the inhibition of photophosphorylation then leads to a chain of subsequent reactions.

In this connection the possibility of a still closer localisation should be mentioned. Calvin and his group (1953) pretend that thioctic acid (synonym: α-lipoic acid) and derivatives play an important role in the early steps of photosynthesis:



from M. Thomas, Plant Physiology, 1956.

From what we know from the chemical behaviour of the triazines an interference by a direct reaction of the 2-chloro derivatives with compounds II and III or by a transesterification in the case of the 2-alkoxy- and the 2-alkylthio derivatives has a certain likelihood and should not be kept out of consideration. In both cases the redox-system would be blocked. My suggestion is, however, completely speculative up to now and not yet consolidated by experimental evidence.

The picture of starch depletion in agropyron repens was studied with special care by Buchholtz (1963; Schirman, R. and Buchholtz, K.P., 1960) and Le Baron from Cornell (1962). After appropriate treatment with Simazine or Atrazine the rhizomes showed up to 98 % reduction of their content of carbohydrates. Buchholtz (1962) claims the limit of 15 % of the regular carbohydrates to be critical so that a reduction of 85 % and more leads to irreversable damage and extinction of the weed.

A contribution which fits very well into this picture of a serious disturbance of the carbohydrate cycle was given by Ashton,F.L. et al.(1960); in red kidney beans exposed in a flat of washed river sand to 1 ppm triazine they found a drastic reduction of the CO_2 fixation, which was practically complete after six hours.

The fact of the inhibition of the Hill reaction induced various researchers to study the behaviour of sensitive plants exposed to triazines in the light and in the dark. Light is indispensable for the morphological symptoms of toxicity (Ashton, F.M., 1962, Allen, W.S. and Palmer, R.D., 1963), and Ashton postulates that chlorophyll is the responsible pigment. Moreland and Hill (1962) hoped to consolidate this hypothesis, when they exposed isolated chloroplasts to various herbicides and washed them afterwards with a solution of Saccharose. When the chloroplasts were kept in the dark, their function was completely restored; when the chloroplasts were exposed to sunlight, then irreversible changes occured. But just in the case of Simazine no significant difference could be observed. The same authors found that chloroplasts of various plant species showed no significant differences in their sensitivity towards triazines and other herbicides which inhibit the Hill reaction; as an example, and this has also been observed in our laboratories, the isolated chloroplasts of the per se highly resistant maize plant are as strongly inhibited as those of sensitive plants. The chloroplasts are consequently not responsible for different sensitivities in the various plant types.

But a repetition of Moreland's chloroplast-reversibility experiments, with other triazines also, would be highly desirable, because Ashton et al.(1963) could see that in bean plants (Phaseolus vulgaris) treated with Atrazine and exposed to light, the chloroplasts of developing and mature leaves are seriously affected. In the course of a 96 hour observation period they ultimately disintegrate; but they remain unchanged in plants placed in the dark with or without Atrazine. The same situation - effect in the light, no effect in the dark - occurs with respect to a reduction of the airspace-system, to a modification of the integrity of the ectoplast and the tonoplast, to a cessation of the cambial activity and a decrease of the thickness of the cell walls of sieve and tracheary elements of the stem. All these changes are attributed by Ashton mainly to secondary intoxication. Ashton together with Uribe (1962) also examined the effect of Atrazine on the sucrose and serine metabolism in red kidney beans. As the changes in the content of alanine and glutamine again do not parallel the ones observed by simple exposure to dark, this is further proof that processes independent of the photosynthetic block must be involved. On the other hand there are also symptoms which are identical in plants treated with Atrazine in the light or which are placed in the dark without Atrazine. In this category figures the acceleration of the vacuolation of cells of developing leaves; this can be considered, therefore, an immediate consequence of the block of the photosynthesis.

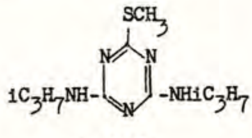
Roth (1958 a), from our laboratories, sees the following sequence of reactions:

The carbohydrates protect catalase against destruction by the light; due to the reduction of carbohydrates as a consequence of triazine interference, the protection ceases and catalase is destroyed. Irreversible damage will therefore occur in plants where catalase is the regulator of the redox-potential necessary for the function of chlorophyll. This, however, is still not a general answer, as Funderburk and Davis (1963) could not find either a correlation of sensitivity to the content of catalase, or to the content of peroxydase, phenoloxydase, ascorbic acid oxydase and glycolic acid oxydase of a plant species. In seven plant species of varying sensitivity tested, the activity of all these respiratory enzymes was reduced under Atrazine-treatment (Funderburk, H.H. and Davis, D.E., 1963). In short term experiments, however, a stimulation of the respiration could be observed (Ashton, F.M., 1960 and Roth, W., 1958 b).

More recently the fact of an increased nitrogen content in some plants after triazine treatment found the interest of various researchers and their studies may give a further contribution to the knowledge of the mode of action. As a special paper (Triazines in top fruit and viticulture by A. Gast and J. Grob) will deal with these aspects at this conference, this phenomenon is only mentioned and not described in detail.

METABOLISM OF PROMETRYNE

After having reviewed and commented upon some studies related to the question "Why and how does the triazine herbicide act?" I would like to make a contribution to the question "Why does a per se phytotoxic triazine compound not act on a given plant species?". This item was discussed in a rather general way at the Symposium for Crop Protection in Ghent early this year, and I should now like to give you some details on the degradation of one representative of the group, namely Prometryne.



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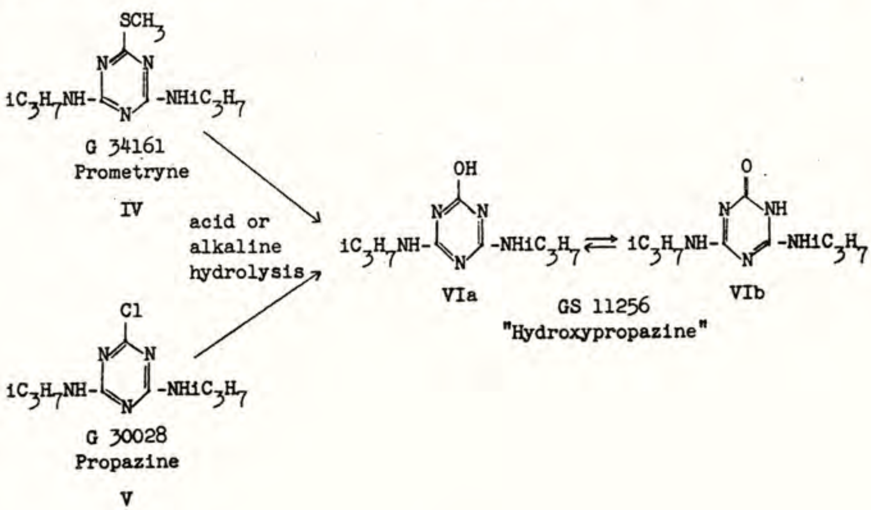
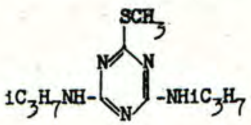
Prometryne

IV

The spectrum of tolerance of Prometryne is different from that of the chlorodiaminotriazines. Maize is more sensitive, cotton, peanuts, peas, carrots and lentils are more tolerant. Prometryne and analogues are more basic than the corresponding chlorodiaminotriazines. Consequently they more easily form salts with acids and the salts are more stable with respect to dissociation in aqueous media. Acid or alkaline in vitro hydrolysis transforms both Prometryne and its chloroanalogue Propazine into the same product 2-hydroxy-4,6-bis-isopropylamino-s-triazine (termed for simplicity's sake hydroxypropazine in the remainder of this report):



With respect to chemical transformation the methylthioderivative offers further possibilities, however, namely those involving oxidative processes. And as oxidation is also very common in the whole mosaic of plant internal biochemical reactions, it was attractive to cast an eye upon the oxidation products of Prometryne. It was possible to synthesize in vitro both the sulfoxy and the sulfono analogue of Prometryne:



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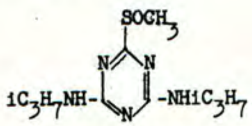
Prometryne
MP 118-120°
IV



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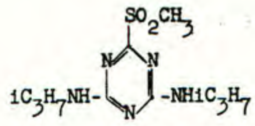

GS 16158
MP 166-177°
VIII

Dr.D.Berrer, 1963.

It was found (Berrer, D., 1963/64) that phthalomonoperacid applied in ethyl ether at -5 to 0°C is the most suitable oxidation agent. The sulfoxide is easily obtained in a good yield, while it is somewhat more difficult to get the pure sulfone. Hydroxypropazine also seems to be a by-product in this oxidative procedure carried out under anhydrous conditions. In a seed germination test at dosages corresponding to about 50 kg/ha both the sulfoxide and the sulfone show practically no phytotoxic response and can, therefore, be claimed as herbicidally inactive.

The study of the hydrolytic behaviour of Prometryne and its two oxidation products was carried out in our analytical laboratories by Delley (1963/64). Hydrolysis of the three compounds yields the same end product, namely Hydroxypropazine. The time-rate of the hydrolysis, as expressed by the half-life value, is given in the following table:

at 25° C	CH_3S	CH_3SO	CH_3SO_2
0,1 n HCl	22 days	96'	16'
H_2O	about 500 years	150 days	3 days
0,01 n NaOH	about 30 years	20'	2'

Delley, R., 1963/64.

From these figures it becomes evident that under acid, neutral and alkaline conditions the sulfone is more quickly hydrolysed than the sulfoxide, i.e. 6 times, 50 times and 10 times more quickly respectively. But the very impressive finding is the enormously increased ease of hydrolytic cleavage of both when compared with Prometryne. A biological system like a plant could, therefore, get rid of this material more easily by oxidation and subsequent hydrolysis than by direct hydrolysis, always with the proviso that biocatalysts would not change the whole picture.

In view of the results of these preliminary in vitro studies it was of great interest to check if the one or the other of these oxidation products could not be found in plants treated with Prometryne. Studies were carried out on peas (pisum sativum) under suitable laboratory conditions by Payot and Müller (1963/64) from our biochemical group. They used Prometryne-C^{14} tagged in the triazine ring, and, after usual working and clean up operations, they ran thin layer chromatogrammes. They were in fact able to detect both the sulfoxide and the sulfone of Prometryne. Further metabolites they could identify were hydroxypropazine and a small but distinct quantity of tagged CO_2, which evidently represents an oxidized ring carbon atom.

These findings can naturally not yet be generalized to other plant species. Also the quantitative side of these reactions is not yet well established; it can be expected that due to the easiness with which they can be hydrolyzed the sulfoxy and the sulfono derivatives will be present, if at all, in minor quantities only. A further question which is still open is the exact manner in which the hydroxytriazines are disintegrated. It is a fact, however, that they are disintegrated. Freed (1962; see also Montgomery, M.L. and Freed, V.H., 1964) fed a whole range of plants with tagged "hydroxyatrazine", non-phytotoxic like all hydroxydiaminotriazines checked up to now, and in all cases he observed a significant development of tagged CO_2, so that the breakdown of the ring is clearly proved.

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SOME ASPECTS OF THE MODE OF ACTION OF SUBSTITUTED PHENOXYACETIC ACIDS

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Summary The pattern and processes of uptake by stem segments provides a system in which the inter-relationships between chemical structure and specific differences at cell level can be investigated. Employing phenoxyacetic acid and its 2-, 4-, 2,4-, 2,6-, and 2,4,5- chloro-derivatives it has been found that irrespective of species, POA and 2-CPA have the highest accumulation and 2,4-D and 2,4,5-T the lowest. In *Gossypium*, but not in *Avena*, an initial phase of accumulation by 2,4-D and 2,4,5-T subsequently is replaced by a phase when there is a net loss to the external solution. It is postulated that there are two mechanisms of accumulation: diffusion followed by metabolic conversion, a reversible equilibrium between entry and egress which can be shifted by the auxin itself or internal processes. Subsequently it was established that the metabolic fate is dependent on the species and compound. In general, POA, 2-CPA, 2,6-D and 2,4,6-T are first hydroxylated and then converted to their β-D glucosides, 4-CPA and 2,4-D may form glucose esters, 2,4,5-T is not metabolised. Hydroxylated compounds are physiologically inactive, glucose esters retain their activity.

Investigating the metabolic conversion of IAA to its aspartate, it has been demonstrated that compounds of high physiological activity, such as 2,4-D, accelerate the rate of conversion, while compounds of low activity (e.g. 3,5-D) do not. In an allied study of the conversion of benzoic acid to benzoyl-aspartate, increased production of the enzymes responsible for conjugation following pretreatment of the tissues with an active auxin is dependent on the synthesis of new messenger RNA.

The implications of these results are discussed.

INTRODUCTION

During the last twenty years thousands of papers have been published which relate to one aspect or another of the physiological effects of natural and synthetic growth regulators. There have been a number of broad approaches which either have sought to link variations in chemical structure with differences in physiological or herbicidal activity or have examined a diversity of physiological and metabolic effects with the view to elucidating specific variations which would allow an appraisal of the mechanisms of selective action.

The criterion of physiological activity which has been widely adopted in investigations of chemical structure has been to measure the effects on extension growth. This approach, although it has proved fruitful in the past, has now reached a stage when no satisfactory theory has been advanced to account for the diversity and complexity of the established facts. This is not surprising. It will be shown later that stem segments cannot be regarded as "simple" systems. In employing the criterion of extension growth the assumption has been accepted that herbicidal activity is a consequence of the effects of supra-optimal concentrations but no clear-cut interactions between chemical structure and the responses of stem tissues of different species have been established in these tests and there is no means of interpreting selective action. Furthermore as several workers have pointed out, there are many other parameters which determine herbicidal action or selectivity which cannot be interpreted without considering mechanisms of uptake by roots, penetration into leaves and transport within the plant.

Turning to the metabolic effects, one is once more faced with a perplexing plethora of assorted data. For example, Woodford, Holly and McCready (1958) list in their review numerous enzymes in a variety of species which are seemingly influenced by 2,4-dichlorophenoxyacetic acid (2,4-D) but nevertheless this wealth of information does not allow one to pinpoint either the basic nature of the toxic action or the reasons for selectivity.

Yet another approach has been to examine the metabolic fate of auxins once they have entered the tissues. Here for indolylacetic acid (IAA) and napthylacetic acid (NAA) the investigations of Andreae and his co-workers (e.g. Andreae, Robinson and Good, 1961), together with those of Zenk, (1962) have established that in segments of pea stem, both auxins rapidly form conjugates with L-aspartic acid and that pretreatment of the tissue with either IAA, NAA or 2,4-D accelerates the rate of conversion. The ability to form such a conjugate is also shared by benzoic acid but compared to IAA and NAA little aspartate is seemingly formed by 2,4-D. In Triticum segments Zenk (1961) and Klämpt (1961) have rigorously established that NAA can form a glucose ester.

Many investigators following Holly, Boyle and Hand (1950) have sought to determine what radioactive products are produced when 2,4-D containing ^{14}C is added to either whole plants or pieces of tissue. For example Luckwill and Lloyd-Jones (1960) found that when leaves of different species were treated with 2,4-D containing ^{14}C in the carboxyl group, the amount of radioactivity lost from the tissues as $^{14}CO_2$ varied considerably between species. However, the general consensus of opinion is that specific differences in the rates of this process of detoxification are too slow to account for selective action. That 2,4-D is capable of forming a number of compounds or complexes in higher plants has been well established by means of chromatographic techniques but up to 1963 there have been speculations but no successes in rigorously identifying any of the compounds formed.

Since this paper relates to the mode of action of substituted phenoxyacetic acids it is only germain to note the investigations of Wain and his research group (vide Wain, 1964) on the varying capacity of species to bring about β-oxidation of the side chain and hence for example a selective action of substituted phenoxybutyric acids.

There is one further comment. A very high proportion of papers have concentrated on the physiological and metabolic effects of 2,4-D and thus there is a paucity of precise comparative information on differently substituted phenoxyacetic acids. The main conclusions that so far can be reached are that besides 2,4-D, 2,4,5-T, 2,4,6-trichlorophenoxyacetic acid (2,4,6-T) and 2-chlorophenoxyacetic acid (2-CPA) can form complexes and that red-currant leaves can decarboxylate 2,4-D, 2,4,5-T, MCPA and 4-chlorophenoxyacetic acid, (4-CPA), but not 2-CPA

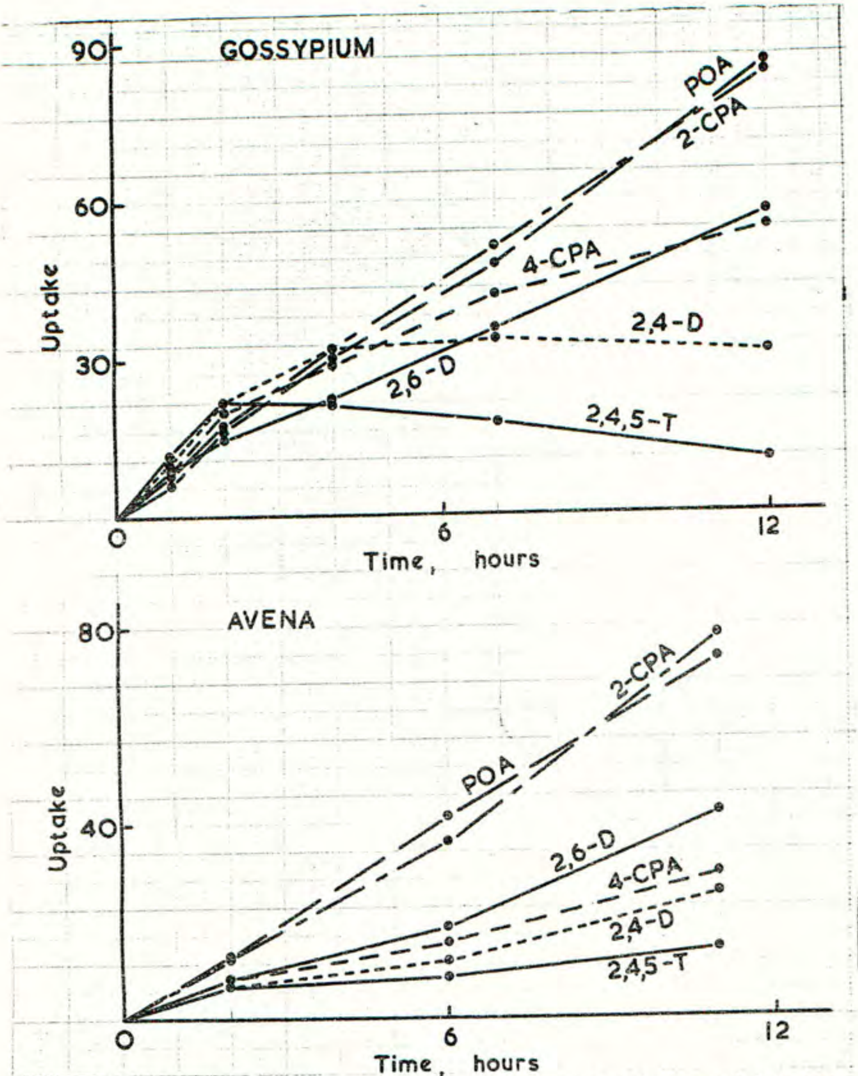
(Luckwill and Lloyd-Jones, 1960).

RESULTS

Against the background of this perforce very truncated summary of previous work, I propose to outline recent and current work in the Unit and in the Department on some basic aspects of the mode of action of some substituted phenoxyacetic acids of varying physiological and herbicidal activities. The first objective is to demonstrate that it is possible to use a single physiological criterion to establish interactions between species and chemical structure. Figs. 1 and 2 illustrate the patterns of uptake of phenoxyacetic acid (POA) and its 2-chloro-, 4-chloro-, 2,6-dichloro-, (2,6-D), 2,4-dichloro- and 2,4,5-trichloro- derivatives by stem segments of Gossypium and Avena. It is to be noted for both species (i) that over the experimental period of 10-12 hours there has been an almost linear accumulation of POA and 2-CPA, (ii) that in Gossypium, 2,6-D and 4-CPA are equally accumulated but to a lesser degree than POA while in Avena, 2,6-D is more readily accumulated than 4-CPA, (iii) that in Avena the lowest rates of uptake are for 4-CPA, 2,4-D and 2,4,5-T but that the rates always remain positive whereas in Gossypium while the initial rates of uptake of 2,4-D and 2,4,5-T are positive, they subsequently become negative. When similar experiments were conducted with segments of Sorghum, Triticum, and Pisum the pattern for Triticum and Sorghum followed that of Avena while the trends for Pisum were more akin to those of Gossypium: for further details see Saunders, Jenner and Blackman (in the press, a).

It will be recalled that in previous studies of the uptake of 2,4-D by Abeyratne (see Blackman, 1961), it was demonstrated that for species which are resistant to herbicidal applications in the field (e.g. Triticum, Avena) uptake by the roots of intact seedlings is an accumulatory process whereas for susceptible species (e.g. Gossypium, Helianthus) an initial phase of uptake is followed by a phase when the 2,4-D "leaks" back into the solution. Thus the old and new evidence together demonstrate that it is possible to select a basis of comparison where there are specific intrinsic differences in the physiological processes involved and that these differences are matched by equally striking divergencies in the effects of compounds of either high or negligible activity as growth regulators or herbicides.

The next point I wish to emphasise is that the processes of uptake and the total content of auxin bear little or no relationship to the magnitude of the increase in extension growth. Firstly in Figs. 1 and 2 whereas the concentration employed of 3 mg/l is about optimal for inducing extension growth by 2,4-D and 2,4,5-T, such a concentration of POA or 2-CPA will have a negligible effect. Secondly, despite controversy, over the first few hours the rate of extension growth of Avena coleoptiles does not change appreciably at concentrations of 2,4-D up to the optimal yet it is clear from Fig. 2 and similar results for coleoptile segments that the amount of growth regulator entering the tissues progressively increases. Thus the rate of extension growth must be influenced by the external concentration rather than by the total internal concentration. To add to the complexity we have now established for all the substituted phenoxyacetic acids cited and for IAA (Südi, unpublished) that for segments of Avena, Triticum, Pisum, Gossypium, entry takes place largely through the cut ends and that for segments of 1 cm. traditionally employed for measurements of extension growth,



Figs. 1 and 2. Comparative courses of uptake expressed as mμ Moles per g fresh wt. of (i) stem segments of Gossypium, (ii) mesocotyl segments of Avena.

an equal distribution of growth regulator along the segment is not attained even after 12 hours. Fig. 3 illustrates the uneven distribution of radioactivity of 2,4,5-T-1-^{14}C in Gossypium segments (1 cm.) after a period of 6 hours: for further details see Saunders, Jenner and Blackman (in the press, b). As a further consequence of entry taking place largely through the cut surface, the uptake of 2,4-D, for example, of twenty 0.5 cm. segments may be nearly double that of ten 1 cm. segments and yet the gain in extension growth as measured by the increase in fresh weight is about the same for equal initial volumes of tissue.

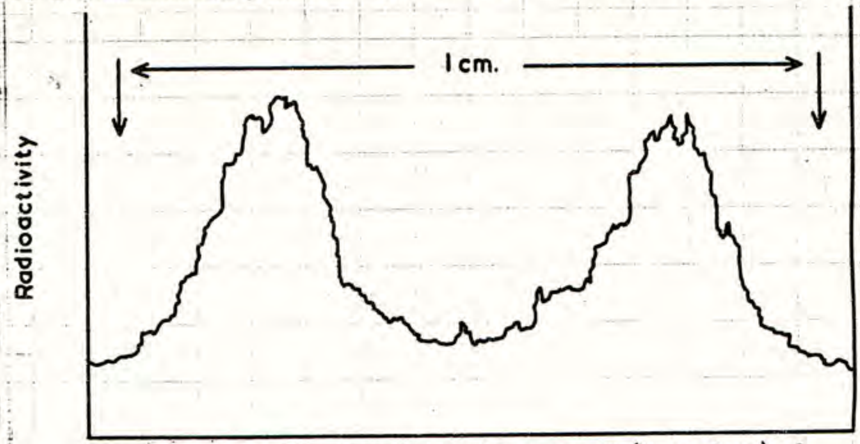


Fig. 3. Distribution of radioactivity along segment (1 cm. long) of Gossypium after 6 hours in buffer containing radioactive 2,4,5-T (15 micromolar).

Lastly, the investigations of Palmer and Loughman (1964) emphasise that major metabolic changes can occur during extension growth. In segments of Pisum the capacity to absorb phosphate varies by twelve-fold within 18 hours of the excision of the segment while for Gossypium and Helianthus there is no change. In parenthesis it should be further noted that for the uptake of phosphate, cut surfaces are not a major pathway of entry.

Much evidence has been accumulated that the courses of uptake shown in Figs. 1 and 2 are primarily determined by metabolic processes but that the processes differ between compounds and between species. By way of illustration, Fig. 4 shows that compared with 2,4,5-T, the uptake of POA is much more sensitive to the inhibitory action of DNP. In contrast, for mesocotyl segments of Avena, to inhibit the initial uptake a much higher concentration of DNP is demanded for POA as against 2,4-D.

The physiological changes during extension growth can also exert an important influence. This is brought out by the results of Fig. 5. Placing segments of Gossypium for 6 hours in buffer alters dramatically the subsequent patterns of uptake and amounts of 2,4,5-T contained in the tissues. When a similar experiment was conducted with coleoptile segments of Avena the curvilinearity of the course of uptake of 2,4-D was reduced by pretreatment in buffer for 13 hours.

The interpretation of these and other results has been discussed by Saunders, Jenner and Blackman (in the press, a and b). Briefly, it is considered that there are two main types of mechanisms involved. In one the growth regulator enters the tissues by diffusion and subsequently is progressively and steadily converted to compounds which are less easily diffusible and so are retained by the tissues. The second type appears to involve a reversible equilibrium between entry and egress and that such a shift can be induced by the growth regulator itself

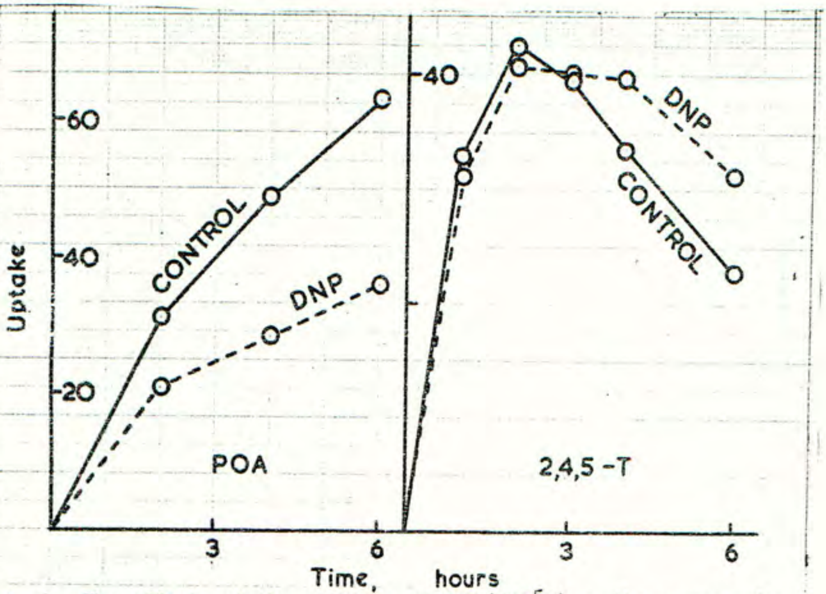


Fig. 4. The effects of the addition of DNP (10^{-5}M) on the course of uptake of POA and 2,4,5-T (15 millemoles) by Gossypium segments. Uptake expressed as mμ Moles per g fresh wt.

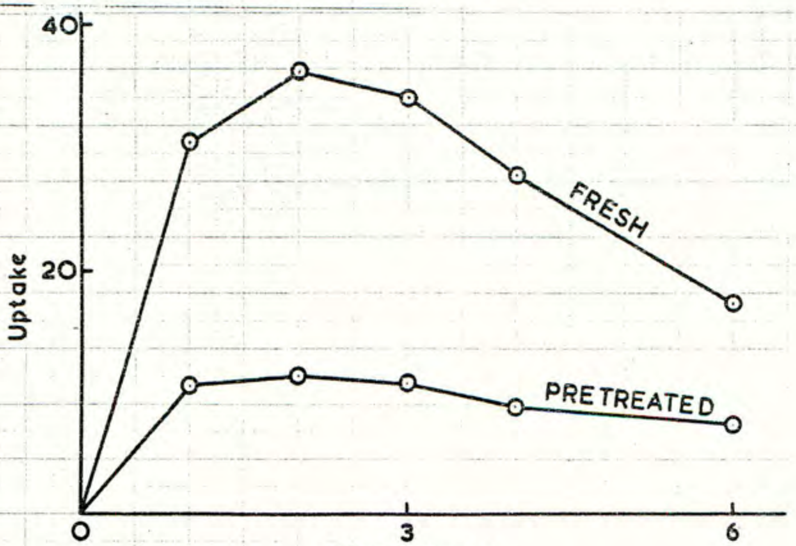


Fig. 5. The course of uptake of 2,4,5-T (15 millemolar) by Gossypium segments, either freshly excised or pretreated in buffer for 6 hours. Uptake expressed as mμ Moles per g fresh wt.

or by internal processes both probably acting on the properties of one or more membranes.

In support of these postulates, Saunders (1963) working with Gossypium and Jenner (1962) with Avena examined the metabolic fate of each growth regulator containing ^{14}C by means of paper chromatography of extracts of the segments using butanol, propionic acid and water (12:5.6:8) as a solvent and a scanning device to determine the distribution of radioactivity. For an experimental period of 6 hours, Jenner concluded that in Avena POA, 2-CPA and 2,6-D were converted into at least two compounds; that 4-CPA and 2,4-D showed some conversion and that 2,4,5-T remained unchanged. For Gossypium segments, by sampling at 2 and 6 hours, it was shown that POA and 2-CPA were converted first into one compound and then into another, that for 2,6-D another compound was detected in small quantities and that for 4-CPA, 2,4-D and 2,4,5-T there was no conversion. Thus these researches established that there were marked interactions between the metabolic fate of individual compounds and the specific nature of the tissues and that compounds which were most actively accumulated were also most readily converted.

The next step was to tackle the difficult problem of identifying rigorously the compounds formed. Most attention has been paid to the metabolic fates in Avena coleoptiles. It was first demonstrated that POA is hydroxylated in the 4-position (Thomas, Loughman and Powell, 1963), and later (Thomas et al, 1964) it was further established that 2-CPA, 2,6-D were also converted to the corresponding 4-hydroxy compound whereas 2,4,6-trichlorophenoxyacetic acid (2,4,6-T) is hydroxylated in the 3-position. Subsequent to hydroxylation 2-CPA is largely converted and accumulated as the 4-O-β-D glucoside while glucosides are also formed by 2,6-D and 2,4,6-T. On the other hand there was no evidence of hydroxylation for 4-CPA, 2,4-D or 2,4,5-T but there was evidence that 4-CPA and 2,4-D formed neutral products of a similar character and that 2,4-D was converted principally to 1-O (2,4-dichlorophenoxyacetyl)-β-D-glucose.

In Triticum hydroxylation was again confined to POA, 2-CPA, 2,6-D and 2,4,6-T but it was clear that not all the species examined had the capacity for this conversion. For example 2-CPA is not metabolised by stem segments of Lupinus albus (Thomas, 1964). Another striking example of specific differences is the fate of 2,4-D in Phaseolus where some conversion to unknown compounds has long been known. It has now been established that a small proportion of the 2,4-D is slowly converted into two compounds: primarily into the glycoside of 2,5-dichloro-4-hydroxyphenoxyacetic acid with smaller amounts of 2,3-dichloro-4-hydroxyphenoxyacetic acid. Thus Phaseolus shares with Aspergillus niger (see Faulkner and Woodcock, 1964) the surprising capacity to shift the position of a chlorine atom in the ring of 2,4-D.

The next questions which clearly arise are what are the physiological significances of these conversions and for the present only tentative answers can be given since the quantities of compounds so far synthesized have not been sufficient for rigorous physiological assessments. On the basis of exploratory tests it would seem that the hydroxylated compounds have little or no activity as growth regulators while the glucose esters of 4-CPA and 2,4-D are active. So it can be tentatively concluded that hydroxylation is a means of detoxification and that the high physiological activity of 2,4-D and 2,4,5-T is associated with their capacity either to remain unchanged or only to be converted into metabolites which are still active.

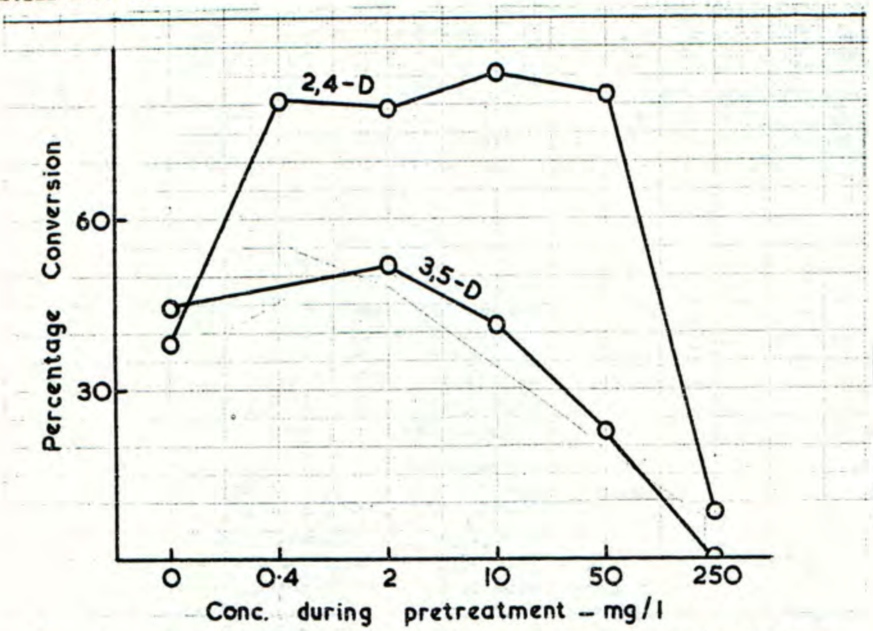


Fig. 6. The effects of concentration during pretreatment for 14 hours with 3,5-D or 2,4-D on the percentage conversion to the aspartate of the IAA in the ethanol extract of Pisum segments after 2 hours of uptake from a concentration of 10 mg/l.

Now I want to switch to another aspect of metabolism, namely the capacity of auxins to induce enzyme activity. In the introductory remarks it was pointed out that IAA and NAA in Pisum segments are largely converted to their respective aspartates and that the initial lag observed in conversion can be eliminated if the segments are first pretreated with IAA, NAA or 2,4-D. This problem has been further investigated in the Department by Südi and a preliminary account has already been published (Südi, 1964). Briefly the results have revealed that the formation of either IAA or NAA aspartate starts immediately if the segments are pretreated with growth regulators of acknowledged high physiological activity, including not only IAA, NAA and 2,4-D but also 2,3,6-trichlorobenzoic acid and S-carboxymethyl-N-N-dimethyldithiocarbamate, whereas compounds of little or no activity as auxins, e.g. 3,5-D, 2,4-dichlorophenoxyisobutyric acid have no effect on the rate of conversion. The differences between 2,4-D and 3,5-D can be gauged from Fig. 6.

According to Andreae and Good, (1955) IAA-aspartate compared to IAA is a very weak auxin while preliminary experiments in this Department suggest that NAA-aspartate is almost inactive. It could be advanced that this loss of activity following conversion is associated with a failure to enter the segments rather than to a lowering of activity at cell level. That penetration does take place has yet to be proven. However, it seems likely on general grounds, and preliminary experiments indicate that the allied 2,4-D aspartate is taken up since it is highly active in promoting extension growth.

If IAA-aspartate within the cell is relatively inactive, then conversion can be regarded as a mechanism for regulating the level of physiologically active IAA. Therefore one of the disruptive effects of 2,4-D and other powerful auxins will be to interfere with this regulatory system.

The other important conclusion that can be drawn from these investigations is that despite the wide differences in chemical structure of the active auxins, they all have the property of inducing a greater rate of aspartate formation. In my limited time, I cannot quote all the evidence but it suggests that the common modus operandi is to bring about an increased production of the enzyme or enzymes responsible for conversion to the aspartate.

The mechanisms by which enzymes are formed or induced are one of the centres of interest of molecular biology but as yet interpretation is a fascinating mixture of fact and speculation. There is, however, general agreement that the primary direction comes from messenger RNA. My closing remarks will therefore be concerned with this aspect.

I have already mentioned that benzoic acid conjugates with L-aspartic acid and Venis, following up Südi's research, has been studying whether pretreatment with compounds other than benzoic acid can bring about greater conversion in *Pisum* segments and what were the conditions which lead to maximal conversion. The results have demonstrated that both IAA and 2,3,6-trichlorobenzoic acid have a greater capacity than benzoic acid to induce its conversion to benzoyl aspartate. It was held (Venis, 1964) that the mode of operation could best be ascribed to the induced formation ofthe enzyme system(s) responsible for the conversion. This being so it was argued that if the production of RNA could be arrested, then no enhancement of aspartate formation would be forthcoming. The tissues were therefore pretreated with IAA alone or IAA combined with actinomycin-D, known to suppress specifically the formation of RNA and puromycin, which arrests protein synthesis and therefore enzyme synthesis. When these two compounds were present during pretreatment, there was subsequently little or no additional formation of the aspartate.

CONCLUSION

By now you will appreciate why the title of this research report was cautiously worded. Clearly there is still much to unravel before the modes of action can be precisely defined but nevertheless pathways through the previous jungle of data can now be seen. It is clear that the accumulation within the cell of differently substituted phenoxyacetic acids is linked both with the structure and specific differences in the processes involved. It is apparent that the metabolic fate is again dependent on the structure and the species and that conversion may or may not lead to inactivation. It has been established that the power to induce the formation of enzymes is also dependent on structure. Within this framework, distinctions can be made between compounds of low and high physiological activity. Lastly, in my opinion, the most important advance is the finding that the induction of enzyme activity is dependent on the synthesis of RNA. It is no longer surprising that there should be such a wide diversity of effects. It opens up the exciting and fundamental prospect of discovering the relationships between auxins and RNA synthesis.

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IOXYNIL - SOME CONSIDERATIONS ON ITS MODE OF ACTION.

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The outstanding herbicidal activity of ioxynil (3,5-di-iodo-4-hydroxybenzonitrile), first discovered at Wye in 1959 (Wain, 1963), led us to make various studies on its mode of action. We have established (Kerr and Wain, 1964a) that ioxynil inhibits the Hill reaction in isolated chloroplasts, being more active in this respect than the mono-iodo or the dibromo derivative and much more active than dinitro-*ortho*-cresol (DNC). 3,5-diiodo-4-hydroxybenzaldehyde, however, is almost as active as ioxynil itself (Fig. 1).

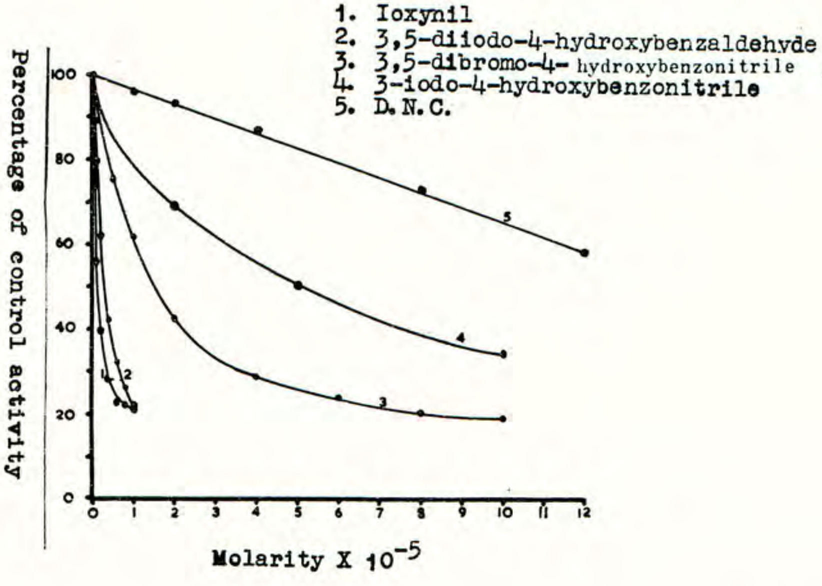


Figure 1. Inhibition of the ferricyanide-Hill reaction by various herbicides.

Another important property of ioxynil is its marked activity in uncoupling oxidative phosphorylation within plant tissues (Kerr and Wain, 1964b). Since other well known uncoupling agents such as DNC and pentachlorophenol possess molluscicidal activity, it was logical to examine ioxynil as a molluscicide. It was found to be highly active in this respect having an LC50 of less than 1 ppm. against two important snail hosts of Schistosomona mansoni, the causative agent of intestinal bilharzia (Wain, 1963). The activity of ioxynil in uncoupling oxidative phosphorylation is about the same as that of DNC as is shown in Fig. 2.

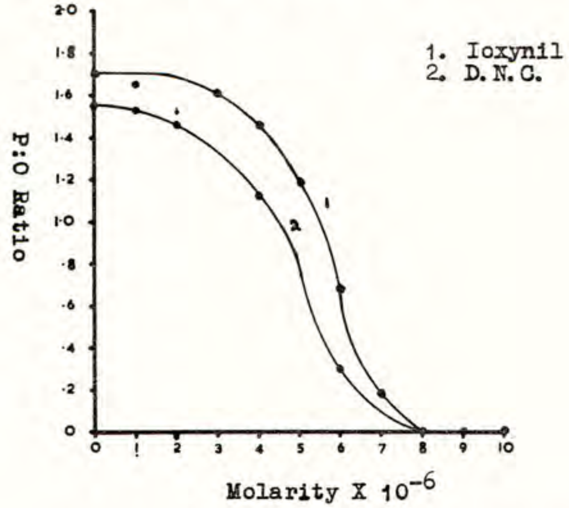


Figure 2. Uncoupling of oxidative phosphorylation by herbicides.

Whilst both of the properties mentioned above may operate in the toxicity of ioxynil to plants, other studies we have made indicate that the mode of action is complex. Thus, it has been established that when applied to plants the compound is considerably more toxic in the light than in the dark. Treated leaves lose their green colour much more rapidly when exposed to light. Similarly, leaf discs placed in aqueous ioxynil solution (1,000 ppm.) lose their chlorophyll in the light but not in the dark.

These and other observations indicate that photochemical reactions are important in the herbicidal action of ioxynil. In this connection my colleague, H. F. Taylor, has established that dilute solutions of ioxynil undergo decomposition when exposed to sunlight (Fig. 3), with the liberation of iodide (Fig. 4).

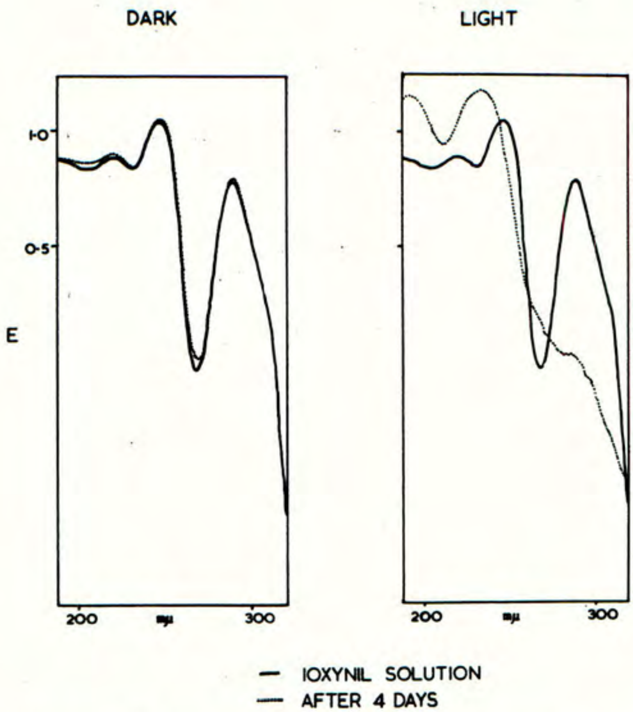


Figure 3. U.V. absorption spectra of ioxynil solution stored in the light and in the dark.

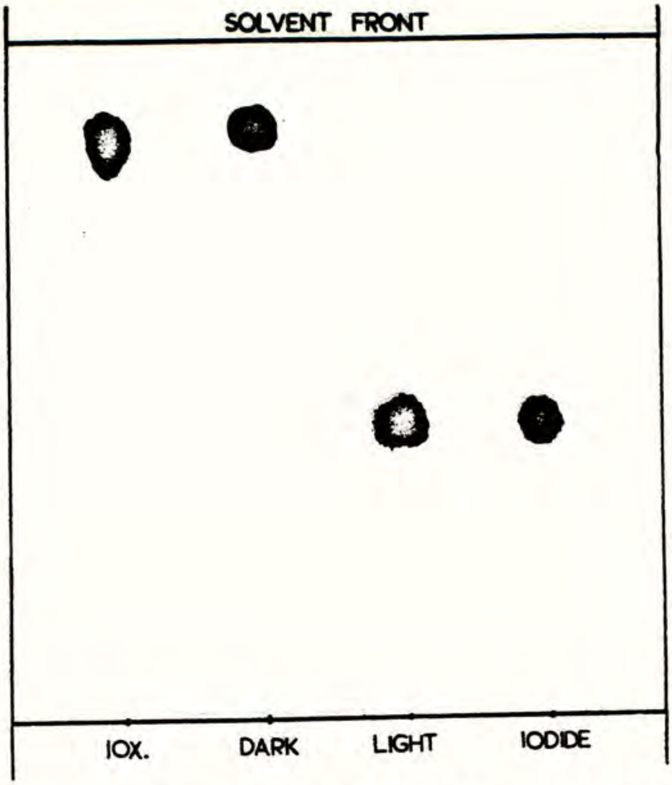
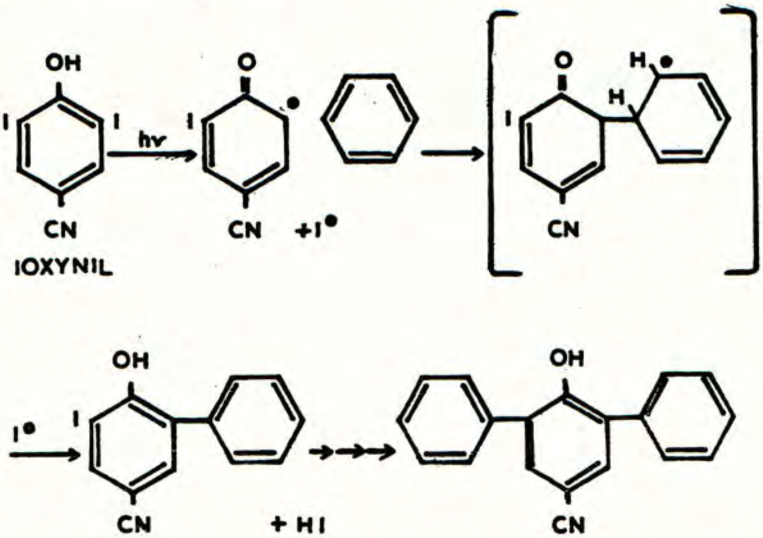


Figure 4 . Chromatogram of ioxynil solutions stored for for 8 days in the light and in the dark. (Paper treated with ceric sulphate/arsenite chromogenic reagent and photograph taken in U.V. light).

Another property of ioxynil is its capacity to combine with benzene when exposed to ultra-violet light. It was shown by Wolf and Kharasch (1961) and Kharasch et al. (1962) that when certain iodobenzene derivatives are irradiated under U.V. light in a suitable aromatic solvent the iodine can be replaced by groups from the solvent. E.N. Ugochukwu has carried out similar photolysis experiments with ioxynil in my laboratory. When irradiated in dry benzene for 20 hours in a specially designed all-quartz apparatus in which the U.V. lamp was surrounded by the test solution and cooling was provided by an outer water jacket, ioxynil was converted to 3,5-diphenyl-4-hydroxybenzonitrile in 71% yield.

There seems to be good reason to believe that this is a reaction involving the formation of free radicals, a possible mechanism being as follows:



The question now arises, do homolytic reactions of this type occur within the tissues of plants treated with ioxynil and exposed to the light? If so, the free radicals liberated within the cells could play havoc with many reactions and processes essential for the life of the plant. It is likely, for example, that free radicals would interfere with the transfer of electrons in the photosynthetic transport system. Investigations on these possibilities are continuing.

It has been shown by H. F. Taylor that when ioxynil is supplied as a solution of its sodium salt at 50 ppm. to bean plants through their roots some translocation of the molecule take place. Breakdown of the compound also occurs. Thus, the corresponding benzoic acid can be detected by chromatography in the stems after five days intake and iodide has also been shown to be present in these tissues. It is not unlikely that further degradation of the molecule with fission of the ring occurs in plants. This raises another possibility in relation to mode of action - that such breakdown might lead to the liberation of simpler yet highly toxic molecules within the cells. One such possibility, although perhaps unlikely, is the production of iodoacetic acid, a potent enzyme poison. This compound is difficult to detect, however, as in aqueous solution it readily undergoes hydrolysis with liberation of iodide.

From the foregoing it is clear that the mode of herbicidal action of ioxynil cannot be simply explained. A study of its properties, however, has already revealed some of the means by which it might exert its phytotoxic effects. A fuller understanding of its mode of action must await the results of further research.

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MODE OF ACTION OF THE BIPYRIDYLIUM HERBICIDES, DIQUAT AND PARAQUAT

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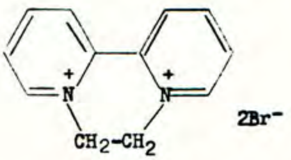
Summary The herbicidal action of diquat and paraquat is dependent upon their reduction in plants to relatively stable, water soluble free radicals. Evidence is presented which suggests that energy for the reduction comes from the primary photosynthetic process and to a lesser extent from respiration. Reduction to the free radical is freely reversible and it is believed that re-oxidation by molecular oxygen of the radicals formed results in the formation of peroxide radicals, or accumulation of hydrogen peroxide which destroys the plant cells.

INTRODUCTION

Diquat is the common name for the 1,1'-ethylene-2,2'-bipyridylium cation manufactured in the form of its dibromide (I) whilst paraquat refers to the 1,1'-dimethyl-4,4'-bipyridylium cation manufactured as the dimethylsulphate or dichloride (II) salt.



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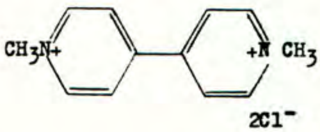


II

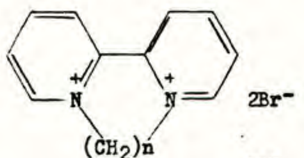
Diquat and paraquat are the active ingredients of the commercial formulations 'Reglone' and 'Gramoxone' respectively. The numerous and varied outlets for the herbicides in world agriculture stems largely from their rapid desiccant action on green plant tissue, when applied at low rates, and their immediate inactivation on contact with soil.

Discovery of diquat at Jealott's Hill Research Station reported by Brian et al (1958) sparked off a programme of work which had as its aim the preparation of quaternary salts derived from the various isomeric bipyridyls. This quickly led to the discovery of the high activity of paraquat and concurrently an examination of the structural requirements conferring high activity within the bipyridyl group. Several workers at Jealott's Hill, notably Drs. Homer, Mees and Tomlinson, were involved in this research programme and the present paper is a survey of their work and of others who have contributed to our present understanding of the mechanism by which these bipyridylium compounds kill plants.

ACTIVITY - STRUCTURE RELATIONSHIP

On assessing the relative herbicidal activity of several of the bipyridylium compounds prepared, certain trends in activity were soon apparent (Homer et al, 1960).

Thus diquat was the only quaternary salt derived from 2,2'-bipyridyl which showed a high degree of activity. Activity was present, though at a reduced level, in the substance with three carbon atoms in the quaternising bridge (III; n=3) whilst the tetramethylene derivative (III; n=4) was inactive. The 'unbridged' dimethyl quaternary salt (IV) of 2,2'-bipyridyl was also inactive.



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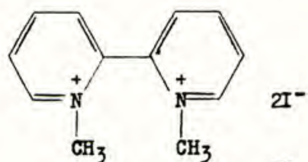
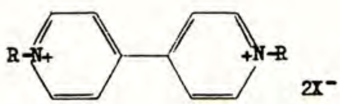


IV

On examining the quaternary salts (V) derived from 4,4'-bipyridyl it was found that activity was present for a wide range of quaternising groups(R), but that all phytotoxic properties were lost by substitution of the ring positions adjacent to the inter-ring bond, e.g. the 3,3'-dimethyl derivatives (VI) were inactive.



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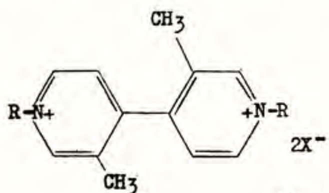
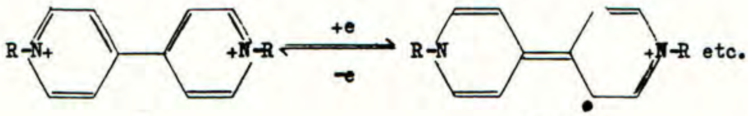


VI

The common factor responsible for high activity thus seemed to be that the molecules must be flat or be capable of assuming a planar configuration. This was supported in the case of the 2,2'-series (III and IV) by molecular models and spectroscopic data (Homer and Tomlinson, 1960).

It was quickly apparent, however, on examining the herbicidal activity of quaternary salts derived from the remaining isomeric biypridyls, that this criterion although necessary was not sufficient. Thus the quaternary salts derived from 2,3'- and 3,3'-bipyridyl were found to be inactive, although capable of a planar configuration.

A clue to a further structural requirement necessary was provided by the earlier work of Michaelis and Hill (1933). These workers showed that quaternary salts of 4,4'-bipyridyl (the 'viologens') were reduced to relatively stable, water soluble, intensely coloured free radicals (VII), formed by the addition of one electron to the diquaternary salt (V) thus:

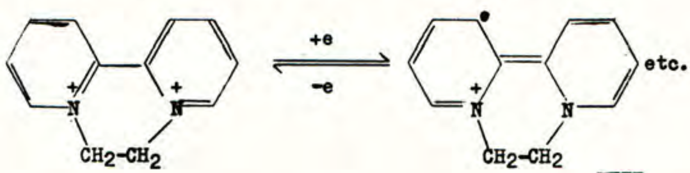


V VII

The free radical formed contains an odd electron and this electron can occupy any of the nuclear carbon positions; in fact eighteen different formulae similar to VII can be written. It is this delocalisation of the odd electron over the whole structure which endows stability to the free radical, which being still a quaternary salt, is water soluble.

This reduction can be demonstrated readily by the addition of a little zinc dust to an aqueous solution of the diquaternary salt. In the case of paraquat the intense blue colour of the free radical quickly develops. Shaking the blue solution with an oxidising agent or in air causes the colour to fade showing that the reaction is reversed by oxidation.

Dr. Homer demonstrated that diquat behaved similarly and formed a green coloured solution of a free radical on reduction. As with the 4,4'- salts, eighteen canonical forms can be written for the radical and the odd electron can occupy any one of the nuclear positions, such as VIII.



VIII

Both groups which have herbicidal activity, the 4,4'- and the bridged 2,2'- salts, are therefore reduced in a similar way. Unless both nitrogen atoms are in positions which are either ortho- or para- to the inter-ring bond, the number of possible resonance forms of the free radical is decreased and, moreover, it is no longer possible to write formulae in which the free electron is located at all of the nuclear positions. This means that the odd electron is not completely delocalised and this reduces the stability of the free radical. The 2,3'- and 3,3'- salts are the ortho-meta' and meta-meta' isomers and are examples of this type. As has been noted already, neither group of salts has herbicidal activity, nor can either group of salts be reduced to free radicals in aqueous solution.

It was also apparent that the condition of coplanarity previously deduced was also a necessary condition for stability of the free radical. Structures such as VII and VIII, requiring the presence of a double bond between the ring, can only exist if the radical is planar. The number of possible resonance forms for a non-planar molecule is thus decreased and consequently the radical is less stable.

In this way we were led to conclude that ease of reduction and phytotoxicity were related and furthermore it seemed a reasonable hypothesis that herbicidal activity depended on the ability of the active compounds to form free radicals by uptake of one electron. There remained a third possible structure for which phytotoxic activity would be predicted according to this theory, viz. salts of 2,4'-bipyridyl where the above-mentioned favourable ortho-para' disposition of the nitrogen atoms is present. The dimethiodide of this isomer, already noted by Krumholtz (1951) to give a purple colour on reduction with the zinc dust was synthesised by Homer (1958) and found to be active. The herbicidal activity was, however, of a much lower order than that of diquat and paraquat. This is probably due to steric hinderance between the N-methyl group of the 2-linked ring and the ortho-hydrogen of the other ring. The much lower redox potential (-640 ± 40mV), see below, of the compound supported this suggestion (Homer and Tomlinson, 1959).

Redox potential is a measure of the ease of reduction of a compound. The higher the negative value of the redox potential the more difficult is the reduction and the free radical formed is correspondingly less stable. The redox potentials of a few 4,4'-bipyridylium quaternary salts, including paraquat, had already been determined by Michaelis and Hill (1933). The redox potentials of diquat and a considerable number of the related bipyridylium quaternary salts were measured by potentiometric titration with sodium dithionite (Homer et al., 1960). It was noted that there was a close relationship between the value of the redox potential and the degree of herbicidal activity, the best herbicides having redox potentials in the range -300 to -500mV. Diquat and paraquat with redox potentials of -349mV and -446mV respectively, fall within this range.

It is postulated that these compounds are active because the applied herbicide is reduced to toxic radicals by a system operating within the plant. Therefore in general, we might expect that the more negative the redox potential, i.e. the more difficult it is to reduce, the less phytoxic it should be. Tomlinson confirmed this, using single leaf dip tests, by measuring the concentration necessary to just kill a plant (threshold concentration). Furthermore, by making use of the fundamental equation for reversible reduction

$$E = Eo - \frac{RT}{F} \log \frac{[Ox]}{[Red]},$$

and by making one or two assumptions, he calculated the concentration of radical [Red] formed from compounds of known redox potential [Eo] at a given potential (E) in the plant. He found that whereas the threshold concentrations [Ox] vary some three hundred fold, the radical concentrations derived from them vary only three fold.

While these results cannot be accepted as proof that free radicals are produced in plants or that these free radicals are the toxic agent, one must nevertheless regard the results with some significance when taken with the whole of the evidence. Much of the confirmatory evidence comes from plant physiological and biochemical studies.

MODE OF PHYSIOLOGICAL ACTION

The physico-chemical evidence presented shows that all the active substances can be reduced to stable, water soluble free radicals by addition of one electron, and the free radicals are converted to the original ion in the presence of oxygen. In order to provide more rigorous evidence that this is the mechanism leading to toxicity it is important to establish (a) the type of reducing system in the plant which might be involved in this re-action and (b) that these compounds are in fact reduced by the biological system.

Mees (1960) first observed that death of plants treated with diquat or paraquat was very much more rapid in the light than in the dark. This can readily be demonstrated in simple experiments with broad bean plants, (*Vicia faba*). If two similar plants have their roots immersed in the same solution of diquat and one plant is kept in the dark it remains apparently unaffected whilst the other plant, whose leaves are in the light, rapidly wilts and dies. As soon as the darkened plant is brought into the light, it too quickly wilts and dies. Plants will eventually die in the dark, but very much more slowly. A further experiment, again due to Dr. Mees, involves a plant with one of its leaves enclosed in a light-tight bag. If a single leaf lower down the plant is immersed in the toxicant and the plant kept in the dark it remains apparently healthy. On bringing the plant into the light it very quickly blackens and dies except for the shielded leaf which remains green and turgid. On removal of the bag and exposure of this leaf to the light it soon blackens like the rest of the plant. Experiments with broad bean leaf discs (Mees, 1960) also show that the rate of herbicidal action, as judged by blackening of the discs, is dependent on the intensity of the incident light.

Only green tissue is affected rapidly in the light. Etiolated shoots which fail to develop chlorophyll in the light after treatment with diquat die much more slowly. From these experiments and others it must be concluded that rapid death of treated plants is associated with photosynthesis.

Another important observation due to Mees was that oxygen is also essential for rapid herbicidal action in the light. Working again with bean discs, he showed that removal of oxygen had an almost immediate effect on the action of diquat in the light. Herbicidal action was almost completely inhibited in an atmosphere of nitrogen. In further experiments he showed that herbicidal activity in the light, as measured manometrically by depression of oxygen uptake, increased as the oxygen tension was increased.

These observations are consistent with the chemical evidence suggesting that reduction of the herbicide to its free radical form is an essential step in the sequence of toxic reactions. Evidence is presented below to show that energy for this reduction comes from photosynthesis in the light and from respiration in the dark. Furthermore, and most important, the action is catalytic, since the reduced form of the herbicide (the free radical) can be re-oxidised by molecular oxygen, thus regenerating the herbicide.

Our present understanding of the mechanism of photosynthesis reviewed recently by Bailey (1963) and National Academy of Sciences (1963) ascribes to illuminated chlorophyll the function of transforming light energy into chemical energy by effecting an electron transfer reaction leading to the formation of reduced pyridine nucleotide ($NADPH_2$) and adenosine triphosphate (ATP). These natural cofactors, energy in the form of ATP and 'reducing power' in the form of $NADPH_2$, are required for the synthesis of sugars from carbon dioxide. Until recently the pyridine nucleotides, with a redox potential of -324mV, were the most electronegative of the electron carrier known to participate in photosynthesis. Tagawa and Arnon (1962) have now isolated from spinach chloroplasts, and crystallised, an iron-containing electron carrier (ferredoxin) with a redox potential of -432mV. It is thus apparent that reducing potentials of the necessary order of magnitude, required for the reduction of the quaternary bipyridylium herbicides, must be generated in green tissue during photosynthesis.

Quaternary salts of 4,4'-bipyridyl under the name of viologens have been used for some time as oxidation-reduction indicators and electron carriers in many types of reaction. The use of the viologens in photosynthesis was first recorded by Horowitz (1952), who found, using heavy oxygen and mass spectrometry, that there was an oxygen uptake when benzyl viologen was added to isolated chloroplasts and illuminated. This is indirect evidence that the viologen is reduced in a photosynthetic reaction.

Jagendorf and Avron (1958) and also Hill and Walker (1959) showed that photosynthetic phosphorylation is catalysed by benzyl and methyl viologen (paraquat salt) when added to chloroplasts and illuminated in the presence of ADP and inorganic phosphate. Davenport (1963) has established the same effect using diquat. These experiments show that the bipyridylium salts are able to act as electron carriers in the system. Many other reducible compounds, such as phenazine methosulphate and vitamin K, are also able to stimulate photosynthetic phosphorylation by illuminated chloroplasts; however, the significance of the reduction of the viologens by chloroplasts is pointed out by both Wessels (1959) and Kandler (1960), namely that they have very negative redox potentials. Wessels considers that they may be reduced by the same mechanism as NADP reduction in photosynthesis.

Using difference spectrophotometry, Kok (1963) and his associates have demonstrated the importance in photosynthesis of a pigment with an absorption band close to 700mμ ("P 700"). This band has been found in brown, red and blue-green algae as well as in green plants. Electron paramagnetic resonance studies have shown that the pigment, "P 700", loses an electron on excitation by high intensity light and remains in a bleached form which exerts the properties of a weak oxidant. It is concluded that the electron acceptor with its additional electron ("X^-") must be a strong reductant of potential lower than -446mV since it reduces methyl viologen (paraquat). Reduction of paraquat to its free radical form was detected by the appearance of its characteristic absorption band at about 395mμ. Kok and Hoch (1963) and Kok, Cooper and Young (1963) were also able to observe limited reduction of the viologen dyes by fresh chloroplasts. The initial rate, as well as the total amount reduced, proved to be highest in light of longer wavelength. The evidence also suggested that the bipyridylium radicals formed on illumination were oxidised by oxygen, or in the absence of oxygen, by the photo-oxidised "P 700".

Apart from this evidence for a light induced reduction of diquat and paraquat in photosynthesis we have clear evidence that diquat and paraquat can be reduced to free radicals during respiration. Thus Turner (1959) has shown a 10-30% stimulation in oxygen uptake by barley roots tips with 10^{-4} - 10^{-5}M diquat and Baldwin (1960) showed that oxygen uptake by cyanide-poisoned yeast could be restored by the addition of diquat. The green colour due to the diquat free radical can, in fact, be readily seen in suspensions of yeast containing diquat kept under anaerobic conditions and paraquat reduction has been observed, by formation of the blue radical, in liquid cultures of certain bacteria. If these suspensions are shaken in air the colour due to the radicals is rapidly discharged.

Although, as yet, we have not demonstrated reduction of diquat and paraquat to free radicals in intact plants there is, as can be seen, a good deal of evidence which supports this indirectly. The bipyridylium quaternary salts must exert their herbicidal effects through reduction and subsequent oxidation, thus replacing or short circuiting the normal redox systems in the plant. The properties which distinguish them from other redox compounds is their reduction by one electron transfer to free radicals and their much lower redox potentials. Reduction of these compounds must occur at the very first stage in either photosynthetic or respiratory electron transfer.

It is considered that a simple replacement or short circuiting of the electron transport chain might result in a slow death due to a depletion in nutrients and energy losses. It is doubtful whether this mechanism alone would account for the rapid and dramatic death of plants which occurs when they are treated with diquat and paraquat in full sunlight. It seems probable that during re-oxidation by molecular oxygen of the free radicals, reactive peroxide radicals are formed or hydrogen peroxide accumulates and it is this which destroys the plant cells.

Some support for this hypothesis has been obtained in the demonstration of peroxide formation during air oxidation of chemically reduced paraquat (Baldwin, 1960). Good and Hill (1955) have also indirectly demonstratedperoxide formation from benzyl-viologen in an illuminated chloroplast suspension using the so-called "Mehler reaction". The hydrogen peroxide was detected by a trapping reaction with excess catalase and ethanol, which gives acetaldehyde and a net oxygen uptake. More recently Davenport (1963) has shown transient peroxide formation by detecting a metmyoglobin-peroxide complex on illuminating a crude chloroplast preparation containing metmyoglobin in the presence of a catalytic amount of diquat.

From the foregoing it is clear that diquat and paraquat are reduced to free radicals by reduction mechanisms operating in chloroplasts or other biological systems and that peroxide radicals or hydrogen peroxide can be formed. Although we still lack the ultimate proof that bipyridylium radicals are formed in the intact plants the evidence is nevertheless compelling. It is possible that further progress can be made by studying the light induced electron spin resonance signals of whole leaf tissue. It may well be that the bipyridylium quaternary salts with their unique properties will provide useful tools for further research into the primary process of photosynthesis.

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Research summary

SOME EFFECTS OF DIQUAT AND SIMETONE ON CO_2-UPTAKE AND TRANSPIRATION OF PHASEOLUS VULGARIS

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Summary The CO_2-uptake of bean leaves was strongly reduced by diquat and simetone sprayed on the leaves, introduced into the petioles, or added to the culture solution, while transpiration was less affected. Diquat was somewhat more active than simetone. In contrast with simetone, this reduction by diquat is followed by a rather gradual development of chlorotic and necrotic spots on the leaves. A combination of simetone and diquat in the culture solution resulted in a more or less additive effect on CO_2-uptake, but largely inhibited the development of chlorotic and necrotic symptoms.

INTRODUCTION

Light is an essential factor in the herbicidal effect of diquat, attributed to the production of toxic free radicals during photosynthesis (Mees 1960). Mees also found inhibition of respiration. In recent studies Davies and Seaman (1964) and Funderburk and Lawrence (1964) have shown that diquat inhibits photosynthesis (O_2-production) of aquatic plants. Also Brian (1964) quotes inhibition of a photosynthetic reaction. In our experiments the CO_2-uptake of leaves of Phaseolus vulgaris was measured after various applications of diquat and compared with that of plants treated with simetone. Also the effect of a simultaneous application of diquat and simetone was studied in view of the inhibition of herbicidal action of diquat by monuron observed by Mees (1960).

MATERIAL AND METHODS

The CO_2-exchange of leaves of Phaseolus vulgaris (var. Beka) in a plexiglass chamber was measured with an infrared gas analyser and recorded continuously. Transpiration was calculated from recorded psychrometer values indicating the humidity of the air. A detailed description of the apparatus was given earlier (Van Oorschot and Belksma 1961), and additional changes are described elsewhere (Van Oorschot, in the press).

After a short dark period to measure respiration and dark transpiration (horizontal dashes in figures) the young plants in the unifoliate leaf stage were subjected to a light intensity of 0.17 cal cm^{-2} min^{-1}. When a constant rate of CO_2-uptake and transpiration was attained, they were treated with purifie non-formulated diquat (as dichloride monohydrate) and/or simet

Since leaf area had been determined previously, they could be sprayed with known rates using a hypodermic needle (with a side arm for air pressure) connected with a syringe and a micrometer. Applications to the roots were made by replacing the normal Hoagland solution by one containing the herbicides. In other experiments, they were introduced into the petioles by means of a stem-puncture technique (Linder and Mitchell 1960). The effects were followed during a period of 15-20 hours. In general the variation between the three replicates was between 5 and 10 per cent.

RESULTS

In experiments with leaf treatments with diquat a sharp decrease in CO_2-uptake (resulting even in negative values after 5 hours), and a comparatively smaller reduction in transpiration were found after spraying an equivalent of 50 g (of the diquat cation) in 200 l. water per ha. A similar rate of simetone was somewhat less effective. On the leaves treated with diquat gradually chlorotic and necrotic spots developed, whereas those sprayed with simetone stayed normal during the experiment. A lower rate (5 g/ha) in other experiments already resulted in 35 per cent decrease of CO_2-uptake and only a few small necrotic spots (necrotic area less than 35%).

Exposure of the roots in the culture solution to a concentration of 2×10^{-5} M diquat also resulted in a sharp decrease of CO_2-uptake (Fig. 1) In experiments at higher light intensity

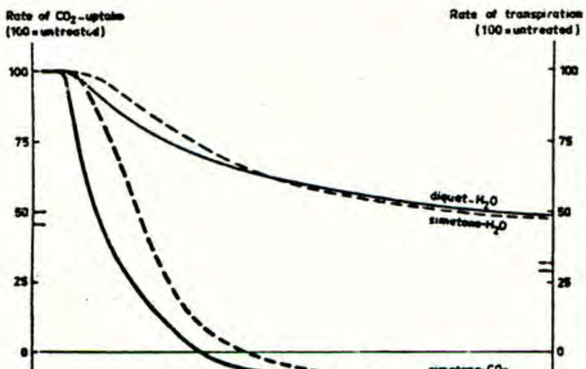

Fig. 1. Effect of diquat and simetone on CO_2-uptake and transpiration of _Phaseolus vulgaris_. At ↑ a culture solution with 2×10^{-5} M diquat or simetone was given. Averages of 3 experiments, leaf temperature during the light period 22.3°C, initial rate of CO_2-uptake (100) 119 μg CO_2 cm^{-2} hr^{-1}, and of transpiration (100) 5.9 mg H_2O cm^{-2} hr^{-1}.

the effect was even more pronounced, while the decrease with lower concentrations was more gradual. In the experiments of Fig. 1 pronounced symptoms (yellow-white bands along the veins) occurred after CO_2-uptake had dropped to values near zero. The equivalent concentration of simetone was again somewhat less effective and the leaves stayed normal.

In other experiments 10^{-8} M diquat (1.84 µg) or simetone (1.97 µg) was introduced in each of the two petioles in 5 µl of distilled water. That of diquat reduced CO_2-uptake to about 1/3 of the initial rate, and that of simetone to about 50%. The introduction of diquat into the petioles gave only necrotic symptoms in a part of the leaves, probably indicating that distribution throughout the leaves was not uniform. The necrotic leaf area was much smaller than would correspond to the reduction in CO_2-uptake. From experiments in which the roots in the culture solution were only for a short period exposed to diquat it is evident that the decrease in CO_2-uptake is irreversible.

The effect of a simultaneous application of diquat and simetone in the culture solution (both at a concentration of 2 x 10^{-5} M) on CO_2-uptake and transpiration is given in Fig. 2, in comparison with that of diquat alone. These results show that the effects of diquat and simetone on CO_2-uptake are more or less additive. On the other hand, the combination of diquat and simetone has less effect on transpiration than diquat alone. Symptoms on the leaves were almost completely absent with this combination.

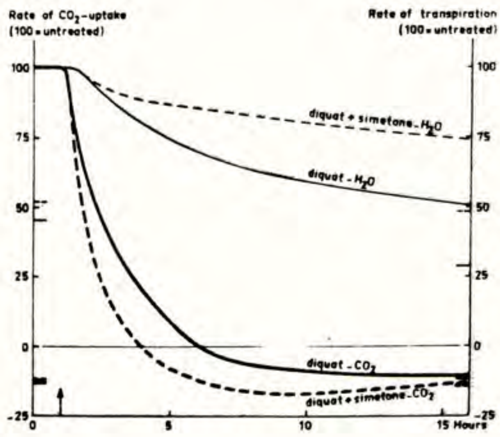

Fig. 2. Effect of a combination of diquat and simetone on CO_2-uptake and transpiration of <u>Phaseolus vulgaris</u> in comparison with that of diquat alone. At ↑ 2 x 10^{-5} M diquat + 2 x 10^{-5} M simetone, or 2 x 10^{-5} M diquat was given to the culture solution. Averages of 3 experiments, leaf temperature during the light period 22.5°C, initial rate of CO_2-uptake (100) 119 µg CO_2 cm^{-2} hr^{-1}, and of transpiration (100) 6.5 mg H_2O cm^{-2} hr^{-1}.

DISCUSSION

The decrease in CO_2-uptake after applications of diquat is in accordance with the inhibition of O_2-evolution of aquatic plants observed by Davies and Seaman (1964) and Funderburk and Lawrence (1964). While the effect of simetone on CO_2-uptake is similar, there is a striking difference in development of symptoms. This development of symptoms seems in accordance with the concept that together with inhibition of photosynthesis toxic free radicals are produced which may disrupt cell membranes (Brian 1964) or chloroplast structure (Lang and Seaman 1964).

As Mees (1960) found with diquat and monuron, and Colby and Warren (1963) with paraquat and solan, the development of symptoms after application of diquat was prevented by simetome. According to Van Overbeek (1964) inhibitors of photosynthesis may reduce formation of toxic free radicals and so prevent herbicidal action of diquat. In our experiments the effects of diquat and simetone on CO_2-uptake were more or less additive. Similar results with diquat and monuron on O_2-production of Lemna were reported by Funderburk and Lawrence (1964). An interpretation of the discrepancy observed between the effect on development of symptoms, and that on inhibition of photosynthesis in intact plants is not well possible.

It is intended to publish a detailed paper on this subject in "Weed Research".

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WEED CONTROL IN HERBAGE SEED CROPS

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Summary Seed of highest genetic purity and freedom from weed seed is primary for quality herbage production. Although cleaning methods to remove weed seed are improving rapidly, elimination of weeds in the field is the most practical method to insure high quality seed. Oregon State University has developed and growers are using a variety of herbicide practices for selective weed removal in seed crops. These include chemical seedbed preparation for more vigorous and weed free establishment. Established stands are treated in the autumn for control of annual grasses such as Italian ryegrass, ratstail fescues, annual bromes, wild oats, annual bluegrass, and perennial grasses such as Yorkshire fog. A variety of herbicides are used including propham, chloropropham, diuron, simazine, atrazine, prometryne, and dicamba. Control of non-grass weeds is accomplished with 2,4-D, MCPA, and dicamba. Research used as a basis for these recommendations is reviewed.

INTRODUCTION

Oregon is the primary producer of a wide variety of the herbage seeds grown in the United States. Unlike many areas, seed is produced as a business rather than a by-product of herbage production. Oregon State University has led in the development of this industry, including the weed control practices, and cleaning equipment needed for quality production.

Seed crops are grown in several climatic regions of Oregon. The marine west Coast climate area west of the Cascade Mountain range produces the greatest variety of herbage seed, but extensive planting of some species are produced in the arid irrigated areas east of the Cascade Mountains.

In the moist mild winter climate region of the West slope, the primary weeds include Italian ryegrass *Lolium multiflorum*, annual bluegrass *Poa annua*, ratstail fescue *Festuca myuros*, silver hairgrass *Aira caryophyllea*, bromes *Bromus rigidus* and *Bromus mollis*, Yorkshire fog *Holcus lanatus*, chickweed *Stellaria media*, mousear chickweed *Cerastium vulgatum*, groundsel *Senecio vulgaris*, sheep sorrel *Rumex acetosella*, dock *Rumex crispus*, mayweed, *Anthemus cotula*, and many others.

In the arid region the primary weeds are annual brome Bromus tectorum, wild barleys Hordeum species, ratstail fescue Festuca myuros, and various non-grass species including the parasitic dodders Cuscuta species.

The major crops grown in Oregon are Italian ryegrass, perennial ryegrass, tall fescue, red fescue, cocksfoot, bentgrasses, and perennial bluegrasses with lesser amounts of Timothy, bromes, and speciality grasses. Legumes include lucerne, white clover, red clover, sainfoin, and annual clovers.

The desire for control of all weed species in seed crops has shifted in recent years to include the seedlings of the crop. This prevents generation shift and maintains genetic purity. Means of controlling crop volunteers from the previous crops during establishment has also assumed major importance with the rapid shift in varieties. Establishment of new plantings without contamination from previous crops of the same species is of considerable value.

The first major work in Oregon on herbicides for selective weed control in herbage seed crops was conducted by V. H. Freed in the late 1940's and early 1950's. Later work has been primarily by W. O. Lee and W. R. Furtick. This paper covers part of the work of these three research workers.

METHODS AND MATERIALS

Experiment 1

This experiment involves the evaluation of herbicides as seedbed preparation aids for control of weeds and volunteer crops in the establishment of perennial grass seed production fields, (Lee, 1964).

A normal fall seedbed was prepared in October on a field that had been fallowed during the summer to kill all perennial grasses. Italian ryegrass was seeded at the rate of 50 pounds per acre to create a heavy volunteer stand. Herbicide applications were made on December 27, 1961; February 1, 1962; and March 7, 1962. The treatments included amitrole, amitrole-T, a dalapon - 2,4-D mixture, diquat, paraquat, and a mixture of propham 2,4-D. On March 23, 1962 seven test crops were seeded in the plots without tillage. The crops used were Italian ryegrass, perennial ryegrass, Merion Kentucky bluegrass, Astoria bentgrass, Pennlawn red fescue, Alta tall fescue, and Potomac cocksfoot. The establishment and vigor of the crops were evaluated in the autumn of 1962. These were compared with the weed control ratings made during the spring to determine the efficiency of the various herbicides in killing the fall germinated species.

Experiment 2

The comparative tolerance of two-year-old stands of perennial ryegrass, red fescue, cocksfoot, and Merion Kentucky bluegrass to several herbicides was determined. Replicated plots were sprayed with two rates each of diuron, simazine, propazine, chloropropham, and endothal on October 2, 1957. A duplicate set of treatments was made on each crop species. Weed control and Yield evaluations were made June 18, 1958 by sampling.

Experiment 3

Several herbicides were applied to one-year-old stands of lucerne, sainfoin, white clover, and red clover to determine relative tolerance and weed control properties under the same environmental conditions. The herbicides applied December 21, 1956 were propham, diuron, and dalapon. Applications made March 25, 1957 were propham, dalapon, and simazine. The weed control and legume injury were evaluated May 27, 1957.

Experiment 4

Autumn trials were established on lucerne in the arid irrigated area. The established lucerne was heavily infested with annual brome Bromus tectorum. Herbicide treatments were made on January 5, 1962. The weed control and lucerne yield as forage were determined on May 2, 1962. This was done by sampling and hand separation of the species.

Experiment 5

The control of annual brome Bromus tectorum in Kentucky bluegrass Poa pratense was determined from the use of dicamba and prometryne. Various timing intervals in October, November, and January were compared. Weed control and harvest yields of seed were used to evaluate the proper time of treatment and dosage.

Experiment 6

Sheep sorrel Rumex acetosella growing in tall fescue was treated with various dosages of dicamba on December 9, 1961. The reduction in live plants was evaluated on July 6, 1962 at the time of harvest.

EXPERIMENTAL RESULTS

Experiment 1

The relative efficiency of the different herbicides or herbicide mixtures used for chemical seedbed preparation was dependent on time of applications. The December treatments gave the best weed control. Only the higher rates of diquat, paraquat, and amitrole-T gave satisfactory control in February, and only paraquat gave control in March. Evaluations of control and seed crop stand made September 18, 1962, (the autumn after treatment) indicated that even in December, amitrole and the dalapon-2,4-D combination were unsatisfactory.

The control data are shown in Table I.

Table I.
Weed control by date of treatment for Herbicides applied in 40 gallons per acre of water

Herbicide	lb/ac	Percent Weed Control December	February	March
paraquat	¼	98	25	7
"	½	99	63	40
"	1	99	85	88
"	1½	99	97	98
"	2	99	99	98
diquat	½	99	7	3
"	1	99	40	10
"	1½	99	63	27
amitrole	1	43	5	0
"	2	96	8	0
"	3	97	10	0
amitrole-T	1	88	10	0
"	2	99	60	0
"	3	99	91	0
dalapon + 2,4-D	5 + 1	40	0	0
propham + 2,4-D	4 + 1	91	99	0

Stand and vigor of grass establishment was directly related to percent weed control. There was no establishment on the untreated check. The establishment was 100% for all species in those treatments that gave 97% or better control. The stand ranged from 0-50% for those with lower percentages of weed control.

Experiment 2

Evaluation of the weed control with various herbicides on four perennial grass species indicated the weedy grasses, primarily ratstail fescue, Italian ryegrass, and Poa annua and non-grasses primarily groundsel Senecio vulgaris and mayweed Anthemus cotula were controlled very well, but injury to the crops varied considerably between herbicides and species. These data are summarized in Table II and III.

Table II.
Weed control from herbicide treatments applied in the Autumn

Herbicide	Lb/ac	Percent weedy grass control	Percent control of non-grass weeds
diuron	1.6	88	94
diuron	3.2	93	100
simazine	1.6	100	100
simazine	3.2	100	100
propazine	1.6	64	96
propazine	3.2	100	100

Table II. cont.

Herbicide	lb/ac	Percent weedy grass control	Percent control of non-grass weeds
endothal	4	0	74
endothal	8	62	98
chloropropham	3	73	44

Table III.
Yield reduction by species from herbicide treatments applied in the Autumn

		Percent yield reduction			
Herbicide	lb/ac	perennial ryegrass	red fescue	blue-grass	cocks-foot
diuron	1.6	42	0	0	0
diuron	3.2	63	78	0	0
simazine	1.6	0	3	20	0
simazine	3.2	12	5	40	10
propazine	1.6	15	0	18	18
propazine	3.2	50	40	48	18
endothal	4	0	0	0	0
endothal	8	10	0	0	0
chloropropham	3	28	0	0	0

Experiment 3

The tolerance of four legumes to herbicides applied in the winter and early spring indicated large differences in tolerance between species. The weed control in general was good. These data are summarized in Tables IV and V.

Table IV.
Weed control from herbicides applied at two dates

			Percent Weed Control	
Herbicide	lb/ac	date applied	grasses	non-grasses
diuron	1.6	December	92	100
diuron	3.2	December	98	100
dalapon	5	December	80	10
dalapon	5	March	72	5
propham	4	December	90	0
propham	4	March	85	0
simazine	3.2	March	95	100

Table V.
Legume Species tolerance to herbicide treatments as percent yield reduction

Herbicide	lb/ac	date applied	lucerne	sainfoin	red clover	white clover
diuron	1.6	Dec.	0	0	0	0
diuron	3.2	Dec.	0	0	15	20

Table V. cont.

Herbicide	lb/ac	date applied	lucerne	sainfoin	red clover	white clover
dalapon	5	Dec.	0	0	85	35
dalapon	5	Mar.	15	0	100	85
propham	4	Dec.	0	0	0	0
propham	4	Mar.	0	0	0	0
simazine	3.2	Mar.	65	15	85	90

Experiment 4

Most herbicides were effective in controlling Bromus tectorum and most non-grass Cruciferous weeds in lucerne without injury when applied in the dormant season. These data are presented in Table VI.

Table VI.
Percent Bromus tectorum control and lucerne yield as affected by herbicide treatment

Herbicide	lb/ac	Control of Bromus tectorum	Tons/ac of lucerne
isocil	.2	100	1.96
isocil	.4	100	1.74
isocil	.8	100	1.63
isocil	1.6	100	1.13*
bromacil	.2	98	1.71
bromacil	.4	100	2.00
bromacil	.8	100	1.85
bromacil	1.6	100	1.27
atrazine	.2	72	----
atrazine	.4	98	1.60
atrazine	.8	100	1.40
atrazine	1.6	100	1.35
Check	0	0	Lucerne 1.52 (Weeds .7)

*Significant at the 5% level of probability

Experiment 5

The control of Bromus tectorum and ratstail fescue by dicamba and prometryne was satisfactory and did not produce significant bluegrass yield differences. These data are given in Table VII.

Table VII.
Percent control of annual brome Bromus tectorum and ratstail fescue with the influence of the treatment on seed yield of Kentucky bluegrass

Herbicide	lb/ac	date applied	Brome control	ratstail fescue control	seed yield
dicamba	2	3 Oct. 1961	70	80	588
dicamba	3	3 Oct. 1961	90	100	527

Table VII. cont.

Herbicide	lb/ac	date applied	Brome control	Ratstail fescue control	Seed yield
dicamba	4	3 Oct. 1961	90	100	411
dicamba	2	17 Jan. 1962	90	90	496
dicamba	4	17 Jan. 1962	100	100	245*
prometryne	2	15 Nov. 1961	80	90	647
prometryne	3	15 Nov. 1961	80	100	488
prometryne	4	15 Nov. 1961	80	100	479
Check	0		0	0	621

*Significant at the 5% level of probability

Experiment 6

The early winter application of dicamba produced a high degree of sheep sorrel kill without injury to tall fescue. The data are presented in Table VIII.

Table VIII.
Sheep sorrel control with dicamba

Herbicide	lb/ac	percent kill
dicamba	1	65
dicamba	2	83
dicamba	4	92
dicamba	8	96

DISCUSSION

The work reported indicates that both perennial grass and legume crops can be selectively weeded through the use of residual soil chemicals applied in the autumn or early winter. Herbicides in the phenyl urea, phenyl carbamate, triazine, uracil, and substituted benzoic acid families may be used for this type of selective control. Crop species and varieties vary considerably in their tolerance to individual herbicides.

Autumn and spring use of dicamba, alone or with 2,4-D or MCPA is effective in controlling many non-grass weeds including the perennials in the genus Rumex. The work reported is only a sampling from a large volume of work conducted over 15 years by three research workers and supplemented by several graduate students. Present work is concentrating on the use of chemical seedbed preparation to insure more rapid and weed free establishment which make the follow-up use of residual herbicides safer and more effective. These combination practices can also control crop volunteers to insure better and longer genetic purity.

Acknowledgments

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References

1. W. O. LEE (1964). The Evaluation of Herbicides as Seedbed Preparation Aids for Control of Weeds and Volunteer Crops in the Establishment of Perennial Grass Seed Production Fields. (Submitted to Weeds).

HERBICIDES AND DESICCANTS IN HERBAGE SEED CROPS IN THE FEDERAL REPUBLIC OF GERMANY

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Summary: This paper reviews the usual methods of weed control in the seed production of grasses and clovers in the Federal Republic of Germany, both in the year of sowing and also in the year of harvest. Results of experiments in the control of monocotyledonous weeds with di-allate and tri-allate are given, as well as a report on the reaction of grass and clover species to several new herbicides. Further problems in grass seed production which are now being worked on are mentioned. The usual method of disiccation of forage seed crops and the results of some experiments are described.

CONTROL OF BROAD-LEAVED WEEDS IN THE YOUNG SEED CROP.

In Germany it is usual to sow grass and clover species destined for seed production under cereal cover crops. This was therefore the subject of the earliest experiments, and their results have already been used for some years in practice.

Grasses which have at least three leaves withstand growth regulators (MCPA; 2,4-D; 2,4-D + 2,4,5-T; CMPP) at the rates which are usual in cereals (Table 1). Individual grass species react differently to growth regulators in depression in growth and production of narrow leaves. But the seed yield in the following year is not influenced. The contact herbicides DNOC, dinoseb and dinoseb-acetate (Table 1) can also be tolerated by young grass seedlings sown under cereals.

The contact herbicides dinoseb and dinoseb-acetate+) can be used in cereals undersown with red clover, white clover, alsike clover and lucerne. The clover plants must have produced at least the first trifoliate leaf at the time of treatment. For the use of dinoseb-acetate, a herbicide which shows practically no residual effect the following possibilities are suggested:

1. Spraying with dinoseb-acetate in winter cereals either just before or up to three days after the sowing of clover.
2. If the weather allows a very early sowing of the clover, probably no weeds would have sprouted in the winter cereal by then and treatment with dinoseb-acetate would have to be delayed until the clover has formed the first trifoliate leaf.

+) 4.6 dinitro-2-sec. butylphenyl acetate

3. If a spring cereal and a clover are sown together, the weeds will probably be susceptible to contact herbicides when the clover is just forming the first trifoliate leaf. In this way dinoseb-acetate can be used with success.

Table 1

Rates of herbicides used in trials with grasses and legumes

herbicides	l/ha or kg/ha active ingredient in grasses	in legumes
MCPA	2.-	1.5 (and 1.-)
MCPB		3.-
CMPP	2.4	
2,4-D	1.4	1.1
2,4-DB		3.-
2,4-D+2,4,5-T	0.9	
DNOC	3.6	
dinoseb	1.2	1.2
dinoseb-acetate	1.6	1.6

In trials by the author with the growth regulators 2,4-DB and MCPB (Table 1) it has been found repeatedly that their herbicidal effect is not sufficient in the conditions which occur in Germany.

As an illustration of how differently herbicides work in different ecological situations, in Germany 1.6 kg/ha dinoseb-acetate is sufficient for weed control in seedling stands, but at least 2.8 kg/ha must be used in Hungary, where the German clover and lucerne breeders propagate their varieties. 2.8 kg/ha can be withstood by lucerne but not by red clover.

Only in special cases e.g. when perennial weeds are present in large quantities, are the usual growth regulators used in Germany in cereals undersown with clover. MCPA is used with red clover and 2,4-D with lucerne.

Experiments with herbicides in other legume species showed that birds-foot trefoil, marsh bird's-foot trefoil and crimson clover are killed to some extent by 2,4-DB, MCPB, dinoseb and dinoseb-acetate, treated in the seedling and young plant stage. Therefore it is not possible to control weeds in stands of these species with the herbicides known today.

CONTROL OF GRASS WEEDS IN THE YOUNG

SEED CROP

In Germany as well as other countries grass weeds have become important, particularly *Alopecurus myosuroides* (blackgrass) and *Avena fatua* (wild oats). These weeds are controlled in cereals by di-allate and tri-allate. The question arose whether di-allate and tri-allate may be used in cereals undersown with grasses.

In 1963 the author tested the response of some grass species to tri-allate. The grasses were sown by hand without a cover crop in 2 cm deep furrows which were marked after spraying with 1.2 and 1.9 l/ha of tri-allate and harrowing. Big differences were found as shown in Table 2. Meadow fescue was particularly resistant. A plant breeder who had treated a field with tri-allate for the control of wild oats before sowing spring barley and meadow fescue confirmed this observation.

Table 2

The response of grasses to di-allate and tri-allate

S = susceptible, MS = moderately susceptible, MR = moderately resistant, R = resistant)

Grass species	di-allate 1.6 l/ha 1964	tri-allate 1.2 and 1.9 l/ha 1963 1.5 l/ha 1964
Fiorin (Agrostis alba)	MS	S
Cocksfoot (Dactylis glomerata)	S	MR
Meadow fescue (Festuca pratensis)	S	R
Red fescue (Festuca rubra)	MS	MS
Italian ryegrass (Lolium multiflorum)	-	MS
Perennial ryegrass (Lolium perenne)	S	MR
Timothy (Phleum pratense)	MS	MS
Swamp meadow grass (Poa palustris)	MS	S
Kentucky blue grass (Poa pratensis)	S	MS
Golden oat grass (Trisetum flavescens)	S	S

In spring 1964 the experiments were repeated. The results agreed with the 1963 observations on the sensitivity of the different types of grass to tri-allate. 1.6 kg/ha di-allate was also tested in 1964. But the resistance of the different types of grass was much less than when treated with tri-allate. For this reason the use of di-allate to control grass weeds is not to be recommended in fields undersown with grass, although it could be used to kill grass volunteers after ploughing up grass seed crops. The extremely dry weather of 1964 showed in addition that the capacity of grasses undersown in barley to resist the herbicides di-allate and tri-allate was considerably reduced by lack of rain. The resistance of some grass species to di-allate and tri-allate as found in these experiments is shown in Table 2. The only grass weed on the experimental field was Poa annua. Germination of this species was prevented at first in the treated plots, but occured later in the season.

USE OF HERBICIDES ON GRASSES IN THE YEAR OF SEED HARVEST

The use of herbicides in the year of harvest has proved successful throughout several years of experiments and has now been put to practice. Seed growers are recommended to spray contact herbicides (particularly

1.6 kg of the mildacting dinoseb-acetate) relatively early in the year, while the weeds are still small. Dinoseb-acetate has the particularly good quality that frost after spraying does not damage the crop.

When growth regulators have to be used, it is advisable to spray after the beginning of the reproductive stage of the grasses, in order to prevent deformities and drop in yield. In Germany most of the grasses reach this stage by the end of April. Some late species such as timothy, swamp meadow grass (Poa palustris), fiorin and also late varieties of perennial ryegrass do not reach the reproductive stage until May. It was found that not only the usual growth regulators (2,4-D; MCPA; 2,4-D + 2,4,5-T; CMPP; Table 1) but also MCPA + TBA used with 12 grass species after the beginning of the reproductive stage, at the doses usual in cereals, did not produce any morphological changes.

A related problem arises from the German practice of sowing clover with the grass which is later to produce seed, with the intention of first taking fodder for one or two years. Before the field can produce grass seed it is necessary to get rid of the clover. Several years of experiments showed that red clover is best killed by 0.9 l/ha 2,4-D + 2,4,5-T, while white clover and alsike clover are most susceptible to 2.4 l/ha CMPP. September of the year before seed harvest is the best time to destroy the clover, before the tiller generation is formed which will bear the seed heads next year. The timely removal of the clover plants growing in competition which the grasses improves grass seed yield. If the clover cannot be killed until the harvest year of the grass seed, then one must wait until the reproductive stage of the grass, but this has the disadvantage of too long a competition between clover and grass, and therefore a smaller yield of grass seed.

Another special problem is the seed production of Westerwolds ryegrass (Lolium multiflorum westerwoldicum). This species of grass is sown without a cover crop, because it already produces seed in the year of sowing. When the first cut is taken for fodder, and the second growth produces seed, then chemical weed control is hardly necessary; the seed yield is however far smaller than it would have been if taken from the first growth. When the first growth is to be taken for seed production, weed control with contact herbicides is safest after development of three leaves. Because this grass species has not reached the reproductive phase when the weeds are in the most susceptible stage, there is a special danger in using growth regulators with Westerwolds ryegrass too early.

CONTROL OF WEEDS IN CLOVER IN THE YEAR OF SEED HARVEST

The control of broad-leaved weeds in clover fields before the harvesting of seed is hardly possible, according to results so far. In the author's experiments the sole chemical which produced no decrease in yield was dinoseb-acetate at the rate of 1.6 kg/ha. The growth regulators based on butyric acid (Table 1) had insufficient herbicidal effect and diminished the seed yield considerably. Even dinoseb diminished the yield. The use of dinoseb-acetate is limited in practice to special cases, because contact herbicides only have a satisfactory effect against young seedling weeds; when weeds are noticed in a thick clover field, they are probably already so advanced, that contact herbicides can at the most only weaken them.

The control of <u>Alopecurus myosuroides</u> in fields of clover and lucerne is, according to Springensguth (196o), possible with 1.5 l/ha CIPC during winter-time.

The author carried out several years of tests on the control of grass weeds growing in clover and lucerne, especially in the control of *Agropyrum repens*. In lucerne 22.5-36 kg/ha TCA and 6.4-12.8 kg/ha dalapon were used and proved fairly effective when sprayed before the beginning of the spring growth or after the first cut. Because both chemicals cause some growth-depression after spraying, it is recommended not to spray after shutting up for seed.

INVESTIGATION OF NEW HERBICIDES

In 1964 the author began experiments with some ureacompounds and triazines on grass and clover species. When this paper was prepared no seed yields were available, but only the field observations (Table 3.). Two year old stands of 13 different grass species were treated with 8 herbicides. Six herbicides were sprayed both at the beginning of spring growth and after the beginning of the reproductive stage. At the first treatment, 1st and 2nd April, most of the grasses were still fully in the vegetative stage; only tall meadow oatgrass, meadow foxtail and Kentucky blue grass were already changing to the reproductive phase. Paraquat was only used at the beginning of spring growth and Banvel-M was only used in the reproductive stage. Comments on the reaction of the grasses are given below:

A. 4 kg/ha A-1114 (50 % prometryne): Eight grasses were partly killed when treated at the beginning of spring growth and in addition showed severe scorch and growth depression after both times of treatment, although after the later treatment all effects were less. In some cases the leaves showed a darker colouring. Seed heads were much reduced in number and size after both treatments. The grass volunteers (seedlings of the crop grasses) were fairly well killed. Awnless bromegrass was almost unaffected, and Kentucky blue grass was very little affected.

B. 4 kg/ha A-1404 (25 % spraying powder of a newer methylmercaptotriazine): The effect on grasses was less than the effect of treatment A. Only in a few cases did killing and severe leaf scorch occur. The effect against volunteers was good. Only in Kentucky blue were seed heads reduced in size and number.

C. 1 kg/ha A-1827 (simazine + prometryne, composition-ratio unknown): The grasses showed in almost every case no lasting after-effect. Unfortunately the control of volunteers was not good.

D. 1 kg/ha A-18o3 (simazine + prometryne, composition-ratio unknown): This treatment caused more damage to the grasses than treatment C, but serious damage is not expected. The control of volunteers was rather better than that of treatment C.

E. 0.8 l/ha paraquat: Partial damage was only observed in Kentucky blue grass and hybrid ryegrass. Early scorching and growth depression were fairly soon overcome. The formation of the seed heads was normal. The effect on volunteers was not encouraging.

F. 3 kg/ha HS 95-1 (Urea-compound from BASF): The effect on the grasses was much greater after the first spraying than after treatment in the reproductive stage. Almost all grasses showed a marked change in habit and appearance, with darker leafcolouring, broader hanging leaves and a greater tendency to lodging. Late meadow grass, fiorin, reed canary grass and Kentucky blue grass were partly killed. Formation of seed heads was, in almost every case, unfavourably affected. The control of volunteers was good.

G. 4 kg/ha H 122 (Urea-compound from BASF): The effect on the grasses was similar to that of treatment F. The same species were damaged and meadow fescue also suffered severely.

H. 4 l/ha Banvel-M: This herbicide is interesting because of the increase of *Matricaria chamomilla* in the crops. When this herbicide was used after the beginning of the reproductive phase the only effect seemed to be a slight change in the appearance of the grass plants, but first results of threshing suggest a possible unfavourable effect on the seed yield.

These herbicides (with the exception of Banvel-M) at the same rates as given above and also 27 kg/ha TCA (I) and 12,8 kg/ha dalapon (K) were tried on lucerne, white clover and red clover. In the same experiments the effect of these chemicals on *Poa annua* was observed. They were sprayed at the beginning of spring growth, and also directly after the first cut.

Lucerne withstood all herbicides well, but after treatment with TCA and dalapon showed some slight and temporary effects.

White clover was badly affected by dalapon (treatment K) and HS 95-1 (F); TCA (I); prometryne (A) and H 122 (G) caused lesser damage; formation of flowers was also affected by A-1404 (B) and A.1803 (D). White clover stood up to paraquat (E) well.

Red clover was very sensitive to TCA and dalapon. Prometryne (A), A-1404 (B) and H 122 (G) caused temporary slight depressions. None of the other chemicals did any harm to red clover.

A good control of *Poa annua* was shown by TCA, dalapon, prometryne (A), paraquat (E) and the urea-compounds (F, G).

VOLUNTEER CEREALS IN GRASS SEED CROPS

Because of the increasing use of combines for the harvesting of cereals, many cereal grains remain on the soil. When grasses have been undersown, the cereal plants growing from these grains crowd out the grass, so that seed yield is considerably diminished. It is hoped to find herbicides which either prevent the cereal volunteers from sprouting, or kill the young cereal plants without affecting the grass. Such experiments have been started.

THOROUGH DESTRUCTION OF OLD SEED CROPS

It is essential to destroy totally the sward of old grass seed crops on farms which specialize in seed production, in order to prevent mixture between different species or varieties. The use of herbicides and of smother crops to suppress the grass regrowth may be successful, and trials have been started.

Table 3

Sensitivity of grass and clover species (stands 2 years old) towards different herbicides in field trials made in 1964 (field observations)

1 = first treatment (at the beginning of spring growth)
2 = second treatment (after the beginning of the reproductive stage)
o = no sensitivity; + = slight temporary damage, ++ = moderate depression
+++ = severe depression, ++++ = very severe damage or kill
rates of A, B, C, D, F, G, H are doses of trade product, rates of E, J, K are active ingredient

	A		B		C		D		E		F		G		H		J		K	
	4 Kg/ha A-1114		4 Kg/ha A-1404		1 Kg/ha A-1827		1 Kg/ha A-1803		0.8 l/ha Paraquat		3 Kg/ha HS 95-1		4 Kg/ha H 122		4 l/ha Banvel-M		27 Kg/ha TCA		12.8 Kg/ha Dalapon	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Fiorin (Agrostis alba)	+++	++	+++	+	o	o	+	+	+		++++	+	+++	+		+				
Meadow foxtail (Alopecurus pratensis)	++	+	o	++	o	o	+	+	o		+	+	+	+		+				
Tall oat grass (Arrhenatherum elatius)	+	+	o	+	+	o	+	o	++		++	+	++	+		o				
Awnless bromegrass (Bromus inermis)	o	+	o	+	o	o	o	o	+		+	o	o	o		+				
Cocksfoot (Dactylis glomerata)	++	+	o	+	++	o	+	o	o		++	+	++	+		+				
Meadow fescue (Festuca pratensis)	+++	++	++	+	o	o	+	o	+		++	++	+++	++		o				
Red fescue (Festuca rubra)	+++	++	++	+	+	o	o	+	++		+	o	+	+		o				
Perennial ryegrass (Lolium perenne)	++	++	o	o	o	o	o	o	+		++	+	+	+		o				
Hybrid ryegrass (Lol. per. + Lol. mult.)	++++	++	++++	++	o	o	o	+	+++		+	+	o	+		+				
Reed canary grass (Phalaris arundinacea)	++	++	o	+	o	o	+	o	o		+++	+	+++	+		+				
Timothy (Phleum pratense)	++	+	o	+	o	o	+	+	+		++	+	++	+		+				
Swamp meadow grass (Poa palustris)	+++	+	++	o	+	o	+	+	+		+++	++	++++	+		+				
Kentucky bluegrass (Poa pratensis)	o	+	++	+	+	+	o	+	+++		+++	+	++	+		+				
Annual meadow grass (Poa annua)	++	+	+	o	o	o	+		+	+++	++	++	++	++			+++	++	++++	++
Lucerne (Medicago media)	o	o	o	o	o	o	o	o	o	o	o	o	+	o			+	+	++	+
Red clover (Trifolium pratense)	+	o	+	o	o	o	o	o	o	o	o	o	+	o			++++	++++	++++	++++
White clover (Trifolium repens)	++	+	+	+	o	+	+	o	o	+	+++	+	++	+			++	+	+++	++

PRE-HARVEST DESICCATION OF CLOVERS, LEGUMES AND GRASSES

The desiccation of clover before seed is harvested is already fairly widespread in Germany. Diquat is used almost without exception.

Because the older methods of harvesting grass seed both take more work and lead to loss of seed, some grass seed crops are also prepared for combine harvesting by killing the green parts of the plants. Without this step it is difficult to thresh the crop while it is still standing.

The author started experiments in desiccation in 1960. At first trials were carried out with a number of chemicals; but only endothal (rates of 0.9 - 1.6 kg/ha) and diquat (0.8 - 1.6 kg/ha) gave good results. Experiments dealt with red clover, white clover, lucerne and different grass species as well as with commom vetch, horse bean and serradella. Unfortunately all chemicals greatly diminished the germination power of serradella. The vetches and clover species retained full germination power. After the use of diquat the grasses suffered in some cases a slight depression in germination capacity; this remained however within a tolerable limit, if not more than 0.8 - 1.2 l/ha diquat were used.

Since 1962 the author has also used paraquat as desiccant in the tests. In most cases the effect was somewhat quicker than with diquat. 0.8 - 1.2 l/ha seemed suitable. The vetches, lucerne, red and white clover showed no depression in germinating power after treatment with paraquat, but serradella and several grass species did (even with 0.4 l/ha paraquat). Therefore paraquat at rates of 0.8 - 1.2 l/ha is only recommended for desiccation of legumes (except serradella), but not in grass seed production.

The author has made many investigations into the moisture-content of the whole crop and of the seed after treatment with desiccants. Moisture of the untreated plots at the time of treatment and at the time of harvest was noted as well as the water content of the treated plots at the time of cutting. Only during very dry weather did the treated plots have slightly less water-content than the untreated ones. When the atmosphere was moist no water-loss was found in the treated plants, and after heavy rainfall the water-content of the treated stands was even higher than in those which were left to wither naturally. The artificially killed plants seem to act like sponges, whilst the still living plants take in less water. For this reason it seems that the name "desiccation" is slightly misleading, in the same way as the German expression "Vorwelke", which also leads one to think of water extraction.

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TRIALS FOR CONTROL OF ALOPECURUS MYOSUROIDES HUDS. (BLACKGRASS) IN GRASS SEED CROPS

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Summary : The trials for control of blackgrass (Alopecurus myosuroïdes) were all carried out on young grass seed crops, in the year of their establishment. Treatments in January, after sowing the preceding September, were generally unsuccessful. Applied in the winter, prometryne and simazine gave rather variable results on spring-sown crops that were at least six months old at the time of spraying ; the poor activity observed at low dosages in Vendée may be due to dry and cold weather following the application, though further investigation should be aimed at determining the best time for treating ; toxicity sometimes observed elsewhere clearly points to a lower degree of selectivity than was assumed when the trials were undertaken. For both triazines the selectivity between blackgrass and the crop is better in spring-sown grasses than in winter wheat, but control needs to be more thorough in grasses than in cereals.

INTRODUCTION

Alopecurus myosuroïdes Huds. (blackgrass or slender foxtail) has become the most widespread grass weed in the arable soils of France. Its presence in a grass seed crop is damaging for two reasons : it reduces the yield of the cultivated plant and it impairs the purity of the harvested seed, which necessitates costly cleaning, supposing the crop has not been rejected by the Commission Officielle de Contrôle which enforces the certification scheme.

The seed producers try to grow the crops in blackgrass-free areas ; but the latter are becoming harder to find, specially as the best soils for seed crops are among those where blackgrass can thrive. Except hand-pulling, cultural means are of little efficacy, particularly during the year of establishment. Chemical weed destruction is consequently important.

First experiments done in France have already been reported (de Gournay, 1964) ; similar work has been done in 1964. There are two groups of crops to consider : spring-sown and autumn-sown. In the latter control is more difficult since crop grass and blackgrass go nearly simultaneously through the same stages in the winter ; but when the crop has been sown in the spring, even under a cereal, it benefits by having more growth and a deeper root system than the weed at the start of the winter. After the year of establishment, even when the field has given one or several seed harvests, blackgrass may infest it again, but dichlobenil (as used against volunteer crop seedlings) can generally destroy it; moreover, if a chemical can be safely applied in the first year, at least after spring-sowing , there is little risk for an older stand in good condition. Research has thus been restricted to blackgrass-infested crops less than one year old.

METHODS AND MATERIALS

Experiment 1 (autumn-sown stand) :

This experiment, at La Minière, near Versailles, involved the following species :

Dactylis glomerata L................	Cocksfoot	Variety	Floréal
Festuca arundinacea Schreb...........	Tall fescue	"	Manade
" " "	" "	"	S 170
Festuca pratensis Huds..............	Meadow fescue	"	Naïade
Lolium hybridum Hausskn.............	Hybrid ryegrass ...	"	Io
Lolium multiflorum Lam..............	Italian ryegrass ..	"	Rina

The six varieties were drilled in September 1963 in contiguous strips (3 m wide for each variety, with an interrow space of 40 cm) alternately with drills of blackgrass to give a row every 20 cm. The treatments were made on January 8 and 9 1964 ; the crop grasses all had from 2 to 10 tillers but the blackgrass had about 30 ; no plant had gone beyond the vegetative stage, except some Italian ryegrasses ; spraying was done across the whole of the strips ; every elementary plot of each variety had an area of 9 m2 and adjoined an untreated control plot ; there were two replicates. A heavy mist set in in the evening of January 8 ; the temperature kept at about 2° C during both days.

The herbicides and the dosages a. i. used were the following :

1.5 and 2.5 kg/ha isopropyl-parachlorocarbanilate ;
2.5 and 4.0 kg/ha sec. butyl-parachlorocarbanilate ;
5.0 and 8.0 kg/ha 5 parachlorophenyl-3 ethyl oxazolidine 2,4 dione ;
5.0 and 8.0 kg/ha 5 parachlorophenyl-3 methyl oxazolidine 2,4 dione ;
3.0 and 5.0 kg/ha of the iminated derivative of parachlorophenylmethane with sec. butylaldehyde cyanhydrine ;
2.5 and 4.0 kg/ha 2,6 dichlorobenzonitrile (dichlobenil) ;
0.8 and 1.2 kg/ha 2 methylmercapto-4 ethylamino-6 gamma methoxypropylamino-s-triazine (GS 12 344).

The last two herbicides were in wettable powders ; the others were formulated as pastes containing 4 to 10 % active ingredient. All were applied by means of knapsack-sprayers with 1,000 l/ha of water ; the mixture in the tanks was kept at 40° C in the case of the first five chemicals in order to avoid crystallisation.

Experiments 2-9 (crops sown in the spring of 1963) :

These experiments in ordinary crops of northwestern France were laid out either as yield trials or as behaviour trials, that is trials performed to test the reaction of the cultivated plant and of the weed without assessing the yield. Details are given in table 1. In all cases the crop had been sown in April or May 1963 under barley ; depression or mortality of crop grass or weed were scored visually as a percentage of the neighbouring control.

The behaviour trials were intended to include simazine at 0.5 and 0.7 kg/ha a.i. and prometryne at 1.0 and 1.4 kg/ha a.i., both at two different dates during the 1963-64 winter, but only one such complete experiment (n° 3) was made.

Table 1.

List of trials carried out in the 1963-64 winter in spring-sown crops

	Trial n°	Month of treatment	Crop species	Location
Behaviour trials	2	Nov & April	Cocksfoot	Manthelon (Eure)
	3	Nov & April	Meadow fescue	Miré (M & L)
	4	Nov	Italian ryegrass	Miré (M & L)
	5	Nov	Tall fescue	St Denis d'Anjou (Mayenne)
Yield trials	6	Nov	Italian ryegrass	Miré (M & L)
	7	Nov	Cocksfoot	Fyé (Sarthe)
	8	Dec	Tall fescue	Chavagnes en P. (Vendée)
	9	Dec	Cocksfoot	Treize-Vents (Vendée)

Trials n° 5 to 7 were blackgrass-free. Crop varieties were Prairial for cocksfoot, Naïade for meadow fescue, Manade for tall fescue and Rina in experiment n° 6 ; in trial n° 4 ryegrass was tetraploïd.

The yield trials were split-plot latin squares. The main plots involved four different dosages (0, I, II and III) ; each was divided in two sub-plots for simazine and prometryne ; application was made with portable sprayers ; beside 0 (control), the dosages were as indicated in table 2. Sixteen m2 or more could be harvested from each sub-plot.

RESULTS

Experiment 1 (autumn-sown crop).

Within a week after treatment all grasses became yellow, probably because of the application of lukewarm water in chilly weather ; they returned to normal colour later.

A) Results for chemicals other than dichlobenil :

In February no important mortality was discernible; in April depressions, all less than 50 %, were observed in some treated plots. The carbamates, the oxazolidines and triazine GS 12,344 gave neither a satisfactory kill of blackgrass nor serious damage to the crop grasses ; the iminated derivative had no visible effect. No important differences in susceptibility between the different species or varieties were seen.

B) Results for dichlobenil :

In April there was a death rate for blackgrass of about 82 % at 2.5 kg/ha and 94 % at 4 kg/ha in the various plots ; but both tall fescues and cocksfoot had suffered more serious damage. Their mortality was more than 90 % at 2.5 kg/ha. Meadow fescue had withstood dichlobenil better than blackgrass, but had been thinned out by 55 % at 2.5 kg/ha, which is not admissible in a crop. The ryegrasses showed a strong depression in February ; in April they were still depressed at 4 kg/ha without being seriously thinned out ; at 2.5 kg/ha they had recovered almost completely.

Experiments 2-9 (spring-sown crops).

A) Behaviour trials :

November applications of prometryne in trials n° 2 and 3 and simazine in trials n° 2 to 4 killed 90 % or more of the blackgrass even at 1.0 and 0.5 kg/ha respectively. Cocksfoot and meadow fescue were not injured by 1.4 kg/ha prometryne, but only cocksfoot tolerated 0.5 kg/ha simazine ; at 0.7 kg/ha of the latter there was in April a depression of 30 % of the cocksfoot and a death rate of respectively 15 % and 98 % of the crop in experiments n° 3 and 4. In trial n° 5 tall fescue displayed a good resistance to both triazines.

Applied in April simazine at Miré and prometryne at Miré and Manthelon destroyed much of the blackgrass within one month in experiment n° 2 and made the weed become completely white in experiment n° 3, but the trials could not be visited later and it is unknown whether the mortality of blackgrass finally proved to be sufficient. Other trials the year before had suggested that April is late for using either triazine against blackgrass in a winter wheat crop in France (de Gournay, 1963).

B) Yield trials :

Two experiments were carried on until harvesting in blackgrass-free soil in order to assess more accurately the depressive effect of triazines and two in moderately infested fields (trials 8 and 9 where the density of the weed was less than 50 plants/m2) to obtain some more data on their activity. Control was good only at the highest dosage of both triazines and, in trial n° 8, at 0.6 kg/ha simazine ; the other dosages killed less than 90 % of the blackgrass.

The crop tolerated the various treatments very well in trials 7 to 9, but in trial n° 6 the crop showed a strong depression in all treated plots in January and three months later, though it displayed only slight damage at the other dosages, its mortality reached 32 % at 0.8 kg/ha simazine and 11 % at 1.6 kg/ha prometryne.

The means of the yield assessments are indicated in table 2.

Table 2

Means of weight measurements in seed yield trials
as percentage of controls

Trial n°	(1)	Dosages (2) I	II	III	Controls (3)	CV (4)
6	P	100.1	108.5	94.1	785	9.8 & 12.1%
	S	95.7	82.8	74.8	821	
7	P	86.2	96.9	91.7	769	16.3 & 15.9
	S	113.6	98.4	103.9	775	
8	P	100.3	108.4	99.7	367	13.4
	S	97.5	96.5	95.1		
9	P	88.7	98.1	92.7	468	11.4
	S	91.0	101.7	89.3		

(1) P = prometryne, S = simazine
(2) Dosages were I = 0.4 kg/ha for simazine and 0.8 kg/ha for prometryne.
II = 0.6 " " " " 1.2 " " "
III = 0.8 " " " " 1.6 " " "
(3) Average yield in kg/ha of controls after cleaning.
(4) Coefficients of variation, the first corresponding to standard error of main plots (dosages), the second to standard error of sub-plots (triazines) ; for trials 8 and 9 analysis could be made only as though they had been designed with total randomization.

In trial n° 6 the yield was significantly decreased at dosage III, even though it was less affected than might have been expected in view of the mortality caused by simazine ; in all four trials there was no other significant difference at the 0.05 level (neither between the dosages, nor between the triazines, and there was no triazine x dosage interaction). However there was at the 0.10 probability level a difference in favour of prometryne in trial 6 and of simazine in trial 7.

DISCUSSION

In 1962-63 oxazolidines and carbanilates applied before tillering had caused great damage to crop grasses and blackgrass ; in 1964, under different experimental conditions, including a much milder winter, they were rather well tolerated by all grasses tested. The irregularity of their activity and their constant lack of selectivity make them unlikely to be suitable for practical use ; any further study does not seem justified, at least in autumn-sown stands. GS 12 344 was disappointing ; but one failure is not enough to give up its testing. Only dichlobenil was somewhat selective between blackgrass and ryegrass, but the study of seedling destruction in grass seed crops has shown that its activity on young grasses depends much upon the time of spraying this feature recalls the effects of barban on wheat and blackgrass. Examining the potentialities of dichlobenil for blackgrass control in ryegrass might initiate an interesting study of plant physiology, but practical results are unlikely : even adult seed crops can be susceptible to dichlobenil when it is applied after November, as shown by trials Bouchet carried out at the Institut Technique des Céréales et des Fourrages (unpublished).

Field experiments performed in 1962-63 had established that simazine and prometryne can give very good blackgrass control in crops at least six months old at the time of spraying. The results described above are more conflicting ; the treatments sometimes failed to come up to expectations against blackgrass ; on other occasions they injured the crop. The failures may be attributed partly to the abnormally dry winter ; also, in Vendée, cold weather brought vegetation to a halt for two consecutive months after the application ; but it is nevertheless important to determine the best time for spraying in the main seed producing regions, which implies more systematical experimentation and particularly more behaviour trials. Injury to the crop was noticed chiefly at Miré, in soils which remain water-saturated throughout the winter ; such soils occur frequently in western France. Simazine and prometryne might profitably be used in grass seed crops in many situations if more were known ; in 1964 simazine at about 0.6 kg/ha gave good control of blackgrass in a number of cocksfoot fields near Evreux, in Normandy, but caused very considerable damage to a young cocksfoot stand in a shallow soil of southern Beauce near Blois. Beside behaviour trials, yield trials are necessary to know which triazine is better under given circumstances.

Simazine and prometryne proved to be, even in 1963-64, much more selective between blackgrass and spring-sown grass seed crops than between blackgrass and winter wheat. Unfortunately, though a 90 % kill of the weed generally suffices in a cereal, one has to be more demanding for ryegrass, cocksfoot or fescue. The problem here is not only to increase the yield of the crop, but also to ensure the cleanliness of the seed ; an example illustrates its difficulty. The blackgrass content of the seed was determined for each plot in trials n° 8 and 9 ; it was always more than might have been expected from the reduction in number of the weeds ; thus 0.8 kg/ha simazine had caused 95 to 98 % of the blackgrass to die in trial n° 8, but the mean rate of blackgrass caryopses in the fescue seed coming from the corresponding plots was only 81 % less than that of the untreated plots. Thinning out the blackgrass increases the tillering of the surviving plants and their seed output when they have not been too heavily depressed by the herbicide. But that alone does not fully explain the disappointing results observed in seed purity ; simazine and prometryne treatments set the blackgrass survivors back more than the crop and probably delayed the shedding of the weed seeds, thus accounting for the harvested seed being more infested than was expected.

The ideal would obviously be to discover a chemical more selective than simazine or prometryne ; TCA does not fill this need for cocksfoot (Robert, 1960) and other grasses ; neither does dichlobenil as it is suitable only for crops already harvested for seed at least once ; dichlobenil is sometimes quite toxic for stands that are only six months old.

Acknowledgments

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Research Summary

GERMINATION OF ALOPECURUS MYOSUROIDES Huds. (BLACKGRASS)

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Summary Alopecurus in winter wheat germinates mainly in late October and early November. Usually 95% of the seeds germinate within 1 year of shedding, but some became dormant when buried deeply or in waterlogged soil. Seeds remain viable longer in dry storage than in moist soil. Light and fluctuating temperatures stimulate germination. Increasing soil-fertility increases the number of seeds per plant but decreases the percent viable seeds. The mechanism of dormancy in Alopecurus has not yet been investigated.

INTRODUCTION

Alopecurus is one of the most abundant weeds on Broadbalk field at Rothamsted where winter wheat has been grown on plots with different fertilisers yearly since 1843. Some information on Alopecurus has been extracted from 30 years' weed surveys and soil-sampling for weed-seed content on Broadbalk. Several small-scale laboratory and glasshouse experiments on Alopecurus seeds collected on Broadbalk have been made since 1961 and the results compared with published data. An outline of the behaviour of Alopecurus in winter wheat has been established but more experiments are needed to elucidate the interaction of factors affecting germination, to see if seeds from other crops and soils behave the same, and to find the causes of dormancy.

RESULTS AND DISCUSSION

Innate dormancy

Seedlings appearing in naturally-infested soil brought into the glasshouse from five plots in 1925 and from the same plots in 1955-61 showed that usually 92 to 97% of the seeds germinate in the first year after shedding and less than 1% survive into the third year. (Brenchley and Warington, 1930; Thurston, unpublished). In Germany, 40% of Alopecurus seeds germinated in the first autumn, none during winter; a second peak of germination occurred in spring (Rademacher, 1959).

Periodicity of germination

The percent of the total seedlings which appeared in each of the four quarters October-December, January-March, April-June and July-September at Rothamsted in 1925 was 83, 7, 3, 7 and for the 7 years 1955-61 it was 82, 10, 1, 7 (Brenchley and Warington, 1930; Thurston, unpublished). Thus, the periodicity had not altered in 30 years and four-fifths of the germination occurred in autumn.

The time of peak germination is shown more accurately by comparing the sowing-dates and infestation of Alopecurus for 30 successive crops of winter wheat. Alopecurus increased in wheat sown in early October, remained at the same level as the previous year when wheat was sown in late October or early November and decreased after late November sowings i.e. most of the Alopecurus seeds germinated between 23 October and 8 November. (Thurston, 1958).

Near Stuttgart, Germany, the number of Alopecurus plants in winter wheat was halved by delaying sowing from 10 October until after 25 October (Rademacher, 1959).

Induced dormancy in waterlogged soil

An unexpectedly heavy infestation of Alopecurus in winter wheat on Broadbalk in 1961 when a wet autumn delayed sowing until January suggested that germination was delayed in very wet soil (Thurston, 1961). Maintaining a water-table at or above the level of Alopecurus seeds sown 1 in. deep in pots of Rothamsted soil induced dormancy. Germination was scarcely increased by allowing free drainage after 3 months' waterlogging, but when drainage was combined with cultivation of the soil to just below the level of the seeds, as many germinated as had previously in pots with free drainage from the start (Thurston, 1962). Lewis (1961) had already demonstrated dormancy of Alopecurus in other waterlogged soils.

Effect of temperature on viability and germination of moist seeds

50% more Alopecurus seeds germinated in sharply-fluctuating than in almost steady temperatures of approximately 60-77°F and seeds kept for 1 year in steady temperatures died (Warington, 1936). Seeds collected 28-31 July and sown in germinators 15 August 1961 germinated only 2% in 30 days at 70°F ± 1°, 60°F ± 1° or 50°F ± 5°. When seeds from 70°F and 60°F were transferred to 50°F, and seeds from 50°F to 60°F, another 6% germinated in 39 days, compared with 1% of seeds kept at their original temperatures. After this all ungerminated seeds were given 70°F for 8 hours followed by 60°F for 16 hours; a further 55% of the seeds germinated in 20 days of alternating temperatures. As this was between 24 October and 13 November i.e. in the normal germination-period of Alopecurus, the increased germination may have been caused by the new temperature-regime or by the age of the seeds. 40% more seeds died when they were held at 50°F for part or all of the first 69 days than at 60°F or 70°F (Thurston, unpublished).

Retention of viability in dry storage

The mean viability of 12 samples collected in 1961 and tested in autumn was 76.5%. The remaining seeds were bulked and stored dry at laboratory temperature; 70% were viable in August 1964 (Thurston, unpublished).

Effect of light on germination

Warington (1936) got 20% more germination in an incubator with subdued light than in a completely dark cellar at approximately the same temperature. Rademacher and Ozolins (1952) agree that light may stimulate germination.

Effect of depth of burying in soil

Seeds originally germinating 61%, buried for 1 year at 9 in., germinated 40.5% when dug up compared with 23.5% from 7 in. and 17.5% from 5 in. (Ling and Price, 1936). The speed of germination when the seeds were brought to the surface varied inversely with depth of burying, indicating that induced dormancy was associated with retention of viability in deeply-buried seeds.

Effect of nutrition of the parent plant on viability and dormancy of seed

Ripe seeds were collected in 1961 and 1962 on an unmanured plot and a plot with complete mineral fertilisers. Germination was tested in germinators under different temperature regimes between August and November, or in sterilised soil in a cool glasshouse where seedlings were counted and removed and the soil was completely broken up and turned over monthly for 3 years. The time of germination and response to temperature were unaffected by the source of seed, but germination from the unmanured plot was 64% in 1961 and 76% in 1962, compared with 57% in both years from the plot with fertilisers (6 cwt/ac sulphate of ammonia, 3.5 cwt superphosphate, 2 cwt sulphate of potash, 1 cwt sulphate of soda, 1 cwt sulphate of magnesia). (Thurston, 1963). This was unexpected because abundant nutrients, especially nitrogen, favour growth of Alopecurus e.g. in 1964 the plot receiving annually 4 cwt/ac sulphate of ammonia plus the other minerals listed above had 3 times as many Alopecurus plants per acre as the unmanured plot and each plant had twice as many inflorescences and 3 times as much dry weight as an unmanured plant. The wheat yield with fertilisers was approximately double the unmanured, so crop competition must have been more severe where fertilisers were given. (Thurston, unpublished). The relative importance of nutrient status and crop competition for seed production and viability in Alopecurus need elucidating.

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CONTROL OF ALOPECURUS MYOSUROIDES (BLACKGRASS) AND OTHER GRASS WEEDS IN GRASS SEED CROPS

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Summary In two seasons' experiments various herbicides were applied to seedling blackgrass and to established crop grasses, to find a treatment for blackgrass in a seed crop's first year. Prometryne $1\frac{1}{2}$ lb/ac merited a tentative recommendation for autumn and winter use, in spite of some disadvantages. Some other less satisfactory treatments and some promising results with new herbicides are discussed. Brief mention is made of the wider application of such treatments to grass weeds other than blackgrass.

INTRODUCTION

An earlier paper (Arthur and Shildrick 1962) outlined the problem of Alopecurus myosuroides Huds. (blackgrass) in grass seed crops, and described autumn use of simazine and prometryne against blackgrass germinating between crop grass rows sown in spring 3 - 6 months before spraying. Simazine as an inter-row band, at $\frac{3}{4}$ - 1 lb/ac of ground sprayed, gave successful results, but an overall treatment would be preferred by most seed growers, provided cost and effectiveness were reasonably similar. Prometryne at $1\frac{1}{2}$ lb/ac was the most promising.

The experiments now described, for the two autumn-to-spring periods 1962 - 3 and 1963 - 4, chiefly tested prometryne more fully, although other overall treatments were included. Treatments requiring incorporation were included in 1962 - 3, but omitted in 1963 - 4 as they increase the cost, need more skill, restrict the time of application and involve the very operation of cultivation which a herbicide treatment is intended to replace.

In 1962 - 3, experiment No. 1 tested 9 herbicides, at up to 4 dates (August to April), on two crop grasses grown and managed like seed crops, with blackgrass seed oversown to give the most realistic possible conditions. Unfortunately the blackgrass did not establish sufficiently to give much information on its control. There were three supplementary experiments. In experiment No. 2, 13 herbicides were applied at a single date to 15 crop grass varieties to indicate whether results for the two varieties in No. 1 could be considered valid for others. In No. 3, 11 herbicides were applied to pure-sown blackgrass before or after emergence, and in No. 4 paraquat and simazine were band-sprayed on blackgrass between rows of perennial ryegrass. These were planned only to give additional information to No. 1, but proved unexpectedly important because of its blackgrass failure.

In 1963 - 4 the single experiment No. 5 was designed to avoid the previous season's difficulties, at the loss of some realism, by growing 11 crop varieties and 4 grass weeds, including blackgrass, as single rows or strips for treatment in October and March with 6 herbicides at 2, 3 or 4 rates.

These five experiments were made on heavy clay on the N.I.A.B. trial ground at Cambridge. Six subsidiary experiments on seed crops on clay in East Anglia were also made during the two seasons, Nos 6-9 in 1962 - 3, and Nos 10 and 11 in 1963 - 4.

METHODS AND MATERIALS

Herbicide treatments and spraying dates. Listed in Tables 1 and 4. 2,6-dichlorothiobenzamide is referred to by the reference number WL 5792.

Application. Knapsack sprayer with reduction valve to give 10 lb. p.s.i. nozzle pressure delivering 40 gal/ac through 6 Dorman No. 2 "no drift" jets mounted 12 in. apart. For band spraying, adjustable lances held to cover full interrow space. For incorporation, rotovation $2\frac{1}{2}$ in. deep, at once after spraying.

Plot size. All 7 ft wide (sprayed width 6 ft). 15-20 ft long when in uniform crop.

Replicates. 3, except 2 in No. 2 and 5 in No. 9.

Details of crop species. (PRG = perennial ryegrass; CK = cocksfoot; MF = meadow fescue; TM = timothy)

Expt.	varieties (all first sprayed in year of sowing except where shown)	Row width (ins)	sown	Height (ins) at spray dates. (cutting to 3 - 4 in.)
1	S143 CK S23 PRG	21 12	22 Mar 62	cut 22 Aug: A:11 B-E:13 A:6 B-E:8
2	S23, S24, S101 PRG; S22 Italian ryegrass; S26, S37, S143, S345 CK; S215, S53 MF; S48, S50, S51 TM; S59 red fescue; S170 tall fescue	14	26 Apr 62	9, after cut 20 Aug
4	S23 PRG	14	2 Aug 62	6
5	S23, S24 PRG; S22 Italian ryegrass; S143, S345 CK; S215 MF; S170 tall fescue; S48 TM; S100 white clover; S123 red clover	21	8 May 63	cut Aug, Sep: A: grass 8, clover 7; cut Dec B: grass 9, clover 3
	S101 PRG	7	Jul 63	A:7 cut Oct B:5
6	S143 CK (in 2nd year)	22	1961	8
7	S215 MF	22	1962	5 (weak, sparse)
8	S53 MF	7	1962	4
9	S37 CK (in 3rd year)	7	1960	11
10	S37 CK	18	1963	4
11	S48 TM	18	1963	A:9 B:10

Approx. age (in months) of crop grasses at spraying given in Tables 1 and 4.

Blackgrass and grass weeds

Expt.

1. Sown over crop by barrow (60 lb/ac) 29 Aug 62, after Date A (28 Aug).

3. Sown in 18 in. band by barrow 30 Aug 62 for date A (sown immediately after spraying and lightly raked in); 27 Aug for date B (2 in. high, 4-5 tillers, at spraying).

4. Sown as No. 1, 27 Aug 62 (2 in. high, 3 tillers at A; 3 in., 6 tillers at B).

5. Agrostis, Bromus mollis sown in rows 30 May 63 } see Table 3.
   Holcus lanatus sown in rows 10 Sep 63
   1st sowing blackgrass sown in band 10 Sep 63 (2-3 leaves at date A: 7 in. at date B)
   2nd sowing blackgrass sown in band 28 Feb 64 (non-em. at date B)

6. Not germinated at spraying: none to record later.

7 & 8. Seedlings (2-4 leaves) to small 3-5 tiller plants, at spraying.

9. Small and medium plants at spraying, later smothered by crop.

10. As 7 and 8.

11. Seedlings to medium plants (6-7 in. diameter) at date A: seedlings (spring-germinated) to small or medium plants at date B.

Records. Crop tolerance and kill of blackgrass and other grass weeds scored by eye; in both respects 9 is the most desirable result and 0 is the least desirable. For brevity, single figures are given, averaging scores for all varieties in an experiment where appropriate. The Tables give dates of recording or intervals between spraying and recording; where two intervals are given above a column the lower one represents the final scoring, and the upper (in brackets) an earlier scoring used to weight the final scores to take account of damage differences at early dates.

Seed yields were only measured for some of the less damaging treatments of S143 cocksfoot in Experiment No. 1. Plots of 4 x 1 yd length (2.3 yd^2) were harvested by hand on 10 Jul, and threshed and cleaned by small equipment to give the clean seed weights shown in Table 2.

RESULTS

The principal summary is Table 1. Comparisons of kill of germinating or seedling blackgrass (central 5 columns) and crop tolerance (right-hand 8 columns) can be made within trials, but less reliably between trials owing to normal variations in scoring. Most treatments achieved good or fairly good control of seedling blackgrass, but the vigorous recovery of any survivors means that only scores of 9 are worthwhile.

TABLE 1

Summary of treatments, spray dates and results in Expts. 1-5.

Herbicide & rate (lb/ac)	Seedling Blackgrass kill (9 = complete : 0 = none) Expt 1 A, spray date 28 Aug 62, not sown, 8 wks	Expt 3 A, 30 Aug 62, not sown, 31 wks	Expt 3 B, 15 Oct 62, Post-em., 24 wks	Expt 5 A, 11 Oct 63, Post-em., 17 wks	Expt 5 B, 23 Mar 64, Pre-em., 10 wks	Crop tolerance (9 = no effect : 0 = kill) Expt 1 A, 28 Aug 62, 5m[ths], (29) 45	Expt 1 B, 27 Sep 62, 6m[ths], (25) 41	Expt 1 C, 14 Nov 62, 8m[ths], (18) 34	Expt 1 D, 27 Feb 63, 11m[ths], (3) 19	Expt 1 E, 19 Apr 63, 13m[ths], 11	Expt 2, 15 Oct 62, 6m[ths], (22) 33	Expt 5 A, 11 Oct 63, 5m[ths], (17) 33	Expt 5 B, 23 Mar 64, 10m[ths], 10
Prometryne 1½			9	9	7		8	8e	8	8e	8	8	9
2				9	8							8	9
3				9	9		7e	8e	8	4	5	7	9
4				9	9							7	9
Barban 5 oz								7e	8				
10 oz				1	0		8e	7e	8		7	8	8
20 oz				3	0							7	7
Paraquat (with wetter) ¼							5	4		5			
¼ B			3 *					8 *					
½							3	2		3			
½ B			5 *					8 *					
1											0		
Simazine ½ B			6 *					9 *					
1	8		9			7e	5	6			3		
1 B	7		8 *			8e	7e	7 *					
Dichlobenil (granules) 2	7 †		9			7e †	6e						
4	9					4	3				1		
Di-allate 2	7 †					8 †	7						
4	8					8	8e				8		
Tri-allate 2	6 †					8 †	8						
4	8					8	8				8		
O.M.U. ½	3	2				8							
2	2	9				8					6		
Diphenamid ½	6	7				8							
2	9	9				7					5		
Simetryne 1			9										
2			9								5		
Ametryne 1			7										
2			9								6		

Continued

B = band application: rate per acre of ground sprayed.

* = Experiment 4: mean of results of band spray dates 9 Oct and 22 Nov 1962 (blackgrass post-emergence: crop grass 2 and 4m[ths]): recorded 33 and 27 weeks after spraying respectively.

† = herbicide incorporated in wide-row cocksfoot.

e = Seed yield result in Table 2.

TABLE 1. (ctd.)

		Blackgrass kill					Crop tolerance							
Experiment		1	3		5		1					2	5	
date		A	A	B	A	B	A	B	C	D	E		A	B
Herbicide														
Desmetryne	½				7	1							9	9
	1			9	8	7							9	9
	2			9	9	8						4	8	9
Amiben	4		9											
WL 5792 (granules)	½				4	7							8	9
	1				8	8							6	7
	2		9	9	8	8							3	5
	4		9	9										
" (spray)	2		9	9										
	4		9	9								1		
Propazine	½				8	8							9	9
	1				9	9							8	9
Linuron	½				3								8	
	1				4	4							8	9
	2				8	7							7	8

TABLE 2. S.143 cocksfoot (Experiment 1)
Tolerance scores and seed yields from some treatments

Herbicide and rate (lb/ac)		Spray date (see Table 1)	Tolerance score (0 - 9 scale)	Seed yield (% of control)
Prometryne	1½	C	8	87
	1½	E	8	83
	3	B	7	81
	3	C	8	73
Barban	5 oz.	C	7	87
	10 oz.	B	8	89
	10 oz.	C	8	68
Simazine	1	A	7	65
" (band)	1	A	7	91
" (")	1	B	6	68
Dichlobenil	2 (incorp.)	A	6	91
	2 (not inc.)	B	6	72
Di-allate	4	B	8	96

Control : 1024 lb/ac clean seed (N.S.)

Crop tolerance was also generally good or fairly good in Nos 1 and 5. In No. 2, the scores show more damage than for comparable rates in No. 1, explicable because the latter's scores were the mean of S23 perennial ryegrass and S143 cocksfoot, which ranked 3rd and 7th respectively among the 15 varieties in No. 2 and so would show a higher mean tolerance than the 15 varieties together. The treatments in Table 1 are compared more fully in the Discussion.

TABLE 3.

Kill of mature blackgrass and other grass weeds (Experiment 5)

(scored for kill : 9 = complete : 0 = none)

		Blackgrass	Agrostis	Bromus mollis	Holcus lanatus
sown		10 Sep 63	30 May 63	30 May 63	10 Sep 63
sprayed		23 Mar 64	11 Oct 63	11 Oct 63	11 Oct 63
recorded		4 Jun 64	10 Feb 64	10 Feb 64	10 Feb 64
Stage at spraying		7 in. high	5 in. high Well-established rows	4½ in. high Well-established rows	2-3 in. high Small plants
Prometryne	1½	0	3	3	9
	2	0	3	5	9
	3	7	5	7	9
	4	7	7	9	9
Barban	10 oz	1	0	0	3
	20 oz	5	0	0	5
Desmetryne	½	0	0	1	7
	1	0	1	3	9
	2	5	5	7	9
WL 5792 (granules)	½	0	5	3	7
	1	2	7	3	9
	2	8	9	5	9
Propazine	½	1	1	0	7
	1	3	5	5	9
Linuron	½	-	1	0	7
	1	0	0	1	9
	2	1	3	5	9

TABLE 4.

Subsidiary experiments (scored as Table 1)

		Blackgrass kill					Crop tolerance						
Experiment No.		7	8	10	11A	11B	6	7	8	9	10	11A	11B
Spraying date		9 Nov 62	23 Nov 62	28 Nov 63	29 Oct 63	6 Apr 64	24 Sep 62	9 Nov 62	23 Nov 62	10 Oct 62	28 Nov 63	29 Oct 63	6 Apr 64
Age at spraying		All seedlings and young plants					18mths	8mths	8mths	30mths	8mths	7mths	12mths
Recorded (wks from spray)		20 32	18 30	10	23	11	(26) 38	(20) 32	(18) 30	(26) 36	10	23	11
Herbicides & rates (lb/ac): Prometryne	1½	9-2	9-9	3	2	1	9	8	8	9	9	9	9
	3	9-5	9-9	4	7	5	9	6	7	8	9	9	9
Barban	10 oz	9-2	9-0					3	2				
Paraquat (with wetter)	¼	9-0	9-0					1	1	4			
	½	9-0	9-0					0	0	2			
Simazine	1	9-9	9-9				9	6	8				
" (band)	1						9						
Desmetryne	1			2	3	3					9	9	9
	2			6	8	6					8	9	9
WL 5792 granules	½			3	2	1					9	9	9
	1			4	7	4					9	7	8

For the three treatments involving incorporation in No. 1, there was no apparent difference between the cocksfoot which had incorporation and the ryegrass which did not.

Table 2 shows the only seed yields which were measured. Although not significant (P = 0.05) all these treatments - though showing only slight to moderate damage in the crop tolerance scores - apparently depressed yield.

The main points from the varietal comparisons in Nos. 2 and 5 were:- 1. Serious damage to S22 Italian ryegrass in both years, showing that the effect was not due only to the severe winter of 1962-3; 2. Several low scores for S59 red fescue in 1962; 3. The tendency of the early-heading S24 perennial ryegrass to score slightly less than the later-heading S23 and S101; 4. A conspicuous resistance by meadow fescue to 4 lb/ac dichlobenil in 1962 and 4 lb/ac WL 5792 in 1963; 5. Greater damage to July-sown S101 perennial ryegrass than to spring-sown S23.

The blackgrass results in Table 1 all relate to pre-emergence or early post-emergence treatments, but Table 3 shows that in experiment No. 5 no treatment was fully effective on mature blackgrass sprayed in spring after establishment the previous autumn, or on mature *Agrostis* or *Bromus mollis*, although seedling *Holcus lanatus* could be controlled.

Table 4 summarizes the subsidiary experiments Nos. 6-11. Initial blackgrass control was good in Nos 7 and 8, but the non-persistence of barban and paraquat prevented final success. Even prometryne gave poor final results in No. 7 because, unlike simazine, it did not control the blackgrass which germinated abundantly in spring owing to the sparseness of the fescue. Blackgrass kill in 10 and 11 was also very poor, almost certainly because some plants were too large at spraying. Paraquat and barban caused serious crop damage, but otherwise tolerance was good except in the weak No. 7 crop.

The severity of the 1962-3 winter must be borne in mind when that season's results are compared with others', but there is no indication that the cold influenced any treatments, except probably on S22 Italian ryegrass. Otherwise, there do not appear to be any results for which meteorological data have special relevance, and these data are therefore not reproduced here.

DISCUSSION

Previous results with prometryne gave it priority in these experiments, and while some treatments were tested nearly as much, for several the results only represent preliminary observations. Table 5 classifies treatments on the results for autumn and winter only in experiments 1-5, omitting the overall paraquat treatments intended mainly to check possible crop damage.

Prometryne at $1\frac{1}{2}$ - 2 lb/ac was better in autumn and early winter than other comparably-tested treatments and probably as good as any in Table 5, while even the overlap rate of 3-4 lb/ac did not cause serious crop damage.

TABLE 5

Tentative classification of treatments on autumn and early winter results from Table 1. (rates in lb/ac).

Crop tolerance	Kill of seedling blackgrass: Good	Kill of seedling blackgrass: Fair	Kill of seedling blackgrass: Poor
Good	Prometryne 1½ Prometryne 2 Propazine 1	Di-allate 2 Di-allate 4 Tri-allate 4 Desmetryne 1 Propazine ½	Simazine (B) ½ Paraquat (B) ¼ Paraquat (B) ½ Tri-allate 2 O.M.U. ½ Diphenamid ½ Desmetryne ½ Linuron ½ Linuron 1 WL 5792 granules ½
Fair or variable	Prometryne 3 Prometryne 4 Diphenamid 2 Ametryne 2 Desmetryne 2 Simetryne 2	Simazine (B) 1 Dichlobenil 2 WL 5792 granules 1 Linuron 2	
Poor	Dichlobenil 4 WL 5792 spray 4	Simazine (overall) 1 WL 5792 granules 2	Barban 5 oz Barban 10 oz Barban 20 oz O.M.U. 2 (conflicting results on blackgrass)
Results uncertain or none	Simetryne 1 Amiben 4 WL 5792 granules 4 WL 5792 spray 2	Ametryne 1	

[B = band]

Some qualifications must be made:-

1. Crop tolerance, though good, was not always complete: the seed yields of Table 2 emphasize this, although 1½ lb/ac gave satisfactory yields in 1961.

2. Treatment is likely to be less effective the older the blackgrass or other grass weed (see Tables 3 and 4), and there is little or no persistency for control of spring germinating weeds.

3. In the limited varietal comparisons which were made, there was greater damage to S22 Italian ryegrass, S.59 red fescue, red and white clovers, and July-sown perennial ryegrass than the other species and varieties tested.

This and earlier work, and the supporting evidence of 1962-3 trials by Messrs Fisons Pest Control, Ltd., (unpublished), led to the following tentative recommendation:-

"Well established seed crops of perennial ryegrass, cocksfoot, timothy, meadow fescue and tall fescue on medium and heavy soils may be sprayed in autumn or winter with 1½ lb/ac prometryne for control of seedling grasses, particularly blackgrass. The grass seed crops should be direct-sown and at least 4 months old. The seedling grasses should have germinated but should not have more than 3-4 leaves. This dose, in spring or on a crop which has not reached the stage described, may cause crop damage; and overlapping should be avoided for the same reason. Italian ryegrass is more susceptible than the species mentioned above. There is no information on rates appropriate for light or high organic soils."

No recommendation is made for spring application because of the damage from 3 lb/ac prometryne in April in Experiment No. 1, but Nos 5 and 11 B suggest that further work may show that spring applications are generally safe, though likely to be effective only against spring-germinated weed grasses.

As in earlier work, simazine at 1 lb/ac overall proved too likely to cause damage. Of the two band spraying rates, $\frac{1}{2}$ lb/ac ground sprayed did not control blackgrass fully, while 1 lb/ac ground sprayed gave some crop damage but less thorough blackgrass control than hoped.

Paraquat as a band-spray was also disappointing at rates used in No. 4, while results from overall application in Nos 1, 7 and 8 illustrate the serious disadvantage of non-persistency, as well as the dangers of inaccurate band-spraying. Experiments, however, by Messrs Plant Protection Ltd., (unpublished), have had encouraging results on grass weeds, particularly Poa species, with band applications of paraquat at $\frac{3}{4}$ lb/ac ground sprayed.

Barban has also given unsatisfactory results, even the high rate of 20 oz/ac proving inadequate for control in the conditions of these trials, as opposed to the success of 5 oz/ac for blackgrass control in cereals.

The other herbicides have not been sufficiently tested for full comparison with prometryne, but further tests on some are being made.

All the treatments considered appear to depend for their effectiveness solely on the differences between crop grass and blackgrass in size, vigour and rooting depth. They can also be used for grass weeds other than blackgrass, or for plants from shed seed of the crop grass, which might affect varietal purity. Other experiments at N.I.A.B. (unpublished) gave good control of timothy seedlings in an established stand of timothy by prometryne ($1\frac{1}{2}$ and 3 lb/ac) and desmetryne (1 lb/ac) on 22 Oct 1963: and of fescue seedlings, Poa species and Bromus sterilis in established meadow fescue by 2 lb/ac prometryne on 28 Aug 1963. One experiment was attempted in 1962 to follow up the most obvious indication of inter-specific difference, by testing simazine ($\frac{1}{2}$ and 1 lb/ac) and paraquat ($\frac{1}{4}$ and $\frac{1}{2}$ lb/ac) for killing Italian ryegrass in perennial ryegrass, but the particular severity of the 1962-3 winter on Italian ryegrass spoilt the experiment.

Acknowledgments

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GROWTH REGULATOR HERBICIDES ON GRASS SEED CROPS PRIOR TO SEED HARVEST

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Summary The current recommendation for growth regulator herbicides on grass seed crops prior to seed harvest advises application in the 4 - 5 weeks before ear emergence. Mecoprop and MCPA/2,3,6-TBA in 1961 and 1962, and dichlorprop and MCPA/dicamba in 1962, with 2,4-D ester for comparison in both years, were applied at rates customary for cereals to seed crops of S.53 meadow fescue and S.48 timothy at five growth stages ranging from about 10 weeks before ear emergence to anthesis. Two stages were earlier than the "safe period", one in it, and two later. Effects were measured by seed yield, germination and seed size. The timothy showed in both years significant yield reductions from spraying at ear emergence, and also in one year from spraying at anthesis and during the safe period. The meadow fescue gave less positive results. Germination was reduced by some treatments. The two MCPA compounds appeared to cause more profound damage than the two phenoxypropionic acids.

INTRODUCTION

Growth regulator herbicides are frequently used on grass seed crops in the spring before seed harvest, to control weeds whose seeds would be difficult to remove from the crop seed. Jeater (1955) found that 2,4-D caused various morphological abnormalities on single spaced plants of four grasses. This and further work in 1955 with 2,4-D and MCPA (Jeater 1958) indicated that the nature and extent of the abnormalities - chiefly in the stems and ears - could be related to the stage of inflorescence development, and it was concluded that grasses grown for seed should only be sprayed with growth regulators after the laying down of the reproductive primordia and before the emergence of the ears from the leaf sheaths. The 4 - 5 week period immediately prior to ear emergence was consequently recommended as a "safe period", varying in calendar date according to species, variety and season. (N.I.A.B. 1957, N.I.A.B. 1962.)

As other growth regulators came to supplement 2,4-D and MCPA, it was generally assumed that their effects would be similar, but in 1961 trials were started to test this. At the same time they would measure seed yields from plants grown as normal seed crops, which Jeater had not attempted to measure on his spaced plants.

Four experiments were made in seed crops on heavy clay near Cambridge. In 1961, experiments 1 and 2 compared mecoprop and MCPA/2,3,6-TBA with 2,4-D ester at 5 growth stages. In 1962, this was repeated in experiments 3 and 4, with MCPA/dicamba and dichlorprop also included. The growth stages aimed at, in the terms used by Jeater (1956), were:-

A. Shoot apex short: primordia vegetative.

B. Apex elongated, with vegetative primordia or at "double ridge" stage of transition to secondary reproductive primordia.

C. Reproductive primordia laid down, and glumes developing.

D. Early ear emergence.

E. Early anthesis (flowering).

Table 1.
Experimental details

Crop Species and variety	Drill width and harvest year	Spray dates*	Approx. interval from 5% ear emergence (- = before, + = after)	Herbicides and doses in oz. a.i./ac	Area harvested in each plot, and date
1961. Experiment 1					
Meadow	14 in.	A 16 Mar	- 10 weeks		6 x 18 in.
fescue	3rd.	B 6 Apr	- 7 weeks		length of row
S.53		C 26 Apr	- 4 weeks		= $10\frac{1}{2}$ ft^2
		D 25 May	5% emerged	2,4-D ester	3 Jul
		E 19 Jun	+ 4 weeks	24	
				Mecoprop	
1961. Experiment 2				40	
Timothy	18 in.	A 6 Apr	- 10 weeks	MCPA/2,3,6-TBA	3 x 1 yd
S.48	3rd.	B 26 Apr	- 7 weeks	12 + 4	length of row
		C 25 May	- 3 weeks		= $13\frac{1}{2}$ ft^2
		D 19 Jun	10% emerged		14 Aug
		E 17 Jul	+ 4 weeks		
1962. Experiment 3					
Meadow	Broad-	A 22 Mar	- 11 weeks		2 x 1 yd
fescue	cast.	B 1 May	- 6 weeks	2,4-D ester	= 18 ft^2
S.53	1st.	C 20 May	- 3 weeks	24	23 Jul
		D 13 Jun	10% emerged	Mecoprop	
		E 29 Jun	+ 3 weeks	40	
				MCPA/2,3,6-TBA	
1962. Experiment 4				12 + 4	
Timothy	24 in.	A 1 May	- 9 weeks	MCPA/dicamba	4 x 1 yd
S.48	1st.	B 24 May	- 5 weeks	18 + 1.3	length of row
		C 5 Jun	- 3 weeks	dichlorprop	= 24 ft^2
		D 29 Jun	7% emerged	40	10 & 13 Sep
		E 24 Jul	+ 4 weeks		

[* A - apex short. B - apex elongated. C - inflorescence developing. D - ear emergence. E - anthesis]

The three first stages were checked in 1961 by microscopic examination of tillers taken at random from the trial area, to verify that the spray dates, often selected to fit weather conditions or other work, coincided reasonably closely with the intended growth stage. It proved difficult, however, to make accurate assessments in the same way as on spaced plants, and as the "safe period" recommendation relates to ear emergence, which can be easily evaluated, this basis for timing was adopted for 1962. (Table 1 gives the percentage of tillers with ears showing above the flag leaf at dates D.) The stage and progress of anthesis is difficult to judge except by frequent visits, but in experiments 1, 2 and 4 it was close to its peak, and in No. 3 it was just starting at date E. Table 1 also shows for each spray date the approximate interval in weeks from early (i.e. about 5%) ear emergence, for comparison with the recommendation on the "safe period". It was intended that only each date C should fall in this period.

METHODS AND MATERIALS

Table 1 summarizes crop details, treatments and spray dates. There were 4 replicates of each treatment, in plots 7 ft by 10 ft, sprayed with a knapsack sprayer fitted with a reduction valve to give 10 lbs p.s.i. at the nozzle, and delivering approx. 40 gal/ac through 6 Dorman No. 2 "no drift" jets mounted 12 in. apart. Experiment 1 was sprayed across the rows, and 2 and 4 with the rows. The boom was held knee-high at the first three stages, but at ear emergence and more particularly at anthesis had to be held higher to ensure that as far as possible the first intersection of the jet cones was just above the tops of the heads.

The plots received the same normal management as the crops they were in, but no herbicides in the year of trial except those applied experimentally.

Seed was harvested by hand from the centres of the sprayed plots (areas and dates in Table 1), and from equivalent areas in twenty unsprayed control plots in each experiment. After harvesting the seed was treated as follows:-

<u>1961.</u> The timothy was tied into sheaves, and left outdoors on a hessian platform to weather for about 3 weeks. The sheaves were turned over occasionally, and turned inside out once. The meadow fescue samples were left hanging in sacks under cover to dry, as was the timothy after weathering. All samples were threshed with a "Garvie" drum-thresher (drum speed 1000 revs/min). The threshed timothy was sieved to remove the straw, and rubbed through a 1 mm wire mesh to break the glumes which were then removed in a mechanical blower at a constant setting. The seed remaining was weighed, after standing in the laboratory for a month to reach moisture equilibrium. The replicates were then bulked and sampled for germination and 1000 seed weight determinations. The fescue samples were cleaned by passing through a 2.5 mm. round hole sieve and being collected on a 6 mm. x 1 mm. wire mesh. They were then put through the blower to remove light seed and chaff, and weighed before the replicates were bulked and sampled for germination, 1000 seed weight and purity determinations. In the fescue, though not the timothy, it was necessary to obtain the pure seed percentage because cleaning had not separated all the light seed: the pure seed percentage was used to adjust the yields to pure seed weights. 1000 seed weights in all cases were based on samples of approximately 500 seeds.
<u>1962.</u> Treatment of the samples after harvest was as in 1961 except that the timothy sheaves were left stooked on the field to weather, and after threshing both timothy and meadow fescue were cleaned through a small "Boby" dresser, the seed being collected under a 3 mm round hole sieve.

RESULTS

Table 2 summarizes the results for 1961. In spite of the differences in yields and a general tendency for them to be reduced by spraying, only the reductions for timothy sprayed at 10% ear emergence were significant, possibly due to the small size of the harvested plots. For 2,4-D and MCPA/2,3,6-TBA these reductions were accompanied by significant loss of germination, while for mecoprop on timothy at the date D there was an increase in 1000 seed weight significant at P = 0.05 (the only significant difference for this character in the two experiments.)

Table 2. Experiments 1 & 2, 1961

	Meadow fescue S.53			Timothy S.48		
Spray Date	2,4-D ester	Mecoprop	MCPA/ 2,3,6-TBA	2,4-D ester	Mecoprop	MCPA/ 2,3,6-TBA
Seed yield as % of control						
A	80	94	86	82	77	119
B	81	76	109	99	94	106
C	120	92	92	83	80	110
D	96	111	109	45**	48**	45**
E	88	79	91	84	82	106
Control		100			100	
		(241 lb/ac pure seed)			(253 lb/ac clean seed)	
		No S.D. at P=0.05			S.D. (P = 0.01)** = 40%.	
Germination %						
A	71	74	77	96	98	96
B		not tested		98	96	96
C	85	79	77	97	96	95
D		not tested		78**	92	77**
E	71	71	60	95	97	94
Control		76			96	
		No S.D. at P = 0.05			S.D. (P = 0.01)** = 18%	

No deformities of leaves or inflorescences were seen in the field. Drought effects were apparent on all plots in late May and early June, but the only field effect of spraying was severe leaf scorch in the timothy from all herbicides at date C.

Tables 3 and 4 summarize the 1962 results. In the meadow fescue in experiment 3 there were no significant differences. Although larger plots were harvested than in 1961, the fescue was completely lodged and this may have introduced enough error in the areas actually cut to prevent the reductions in yield and germination on the date E achieving worthwhile significance.

In the timothy in experiment 4, however, many of the yield reductions were very significant, which may be linked to the larger plots and the fact that the crop was standing and could be more accurately measured for cutting. For all herbicides, there were also increases significant at

P = 0.01 in 1000 seed weight at date D, indicating compensation by the plants for reduced numbers of fertile heads or florets. In neither experiment were any deformities or other effects such as scorching observed in the field.

Table 3. Experiment 3, 1962 (Meadow fescue S.53)

Spray Date	2,4-D ester	Meco-prop	Dichlor-prop	MCPA/ 2,3,6-TBA	MCPA/ dicamba
Seed yield as % of control					
A	122	132	- @	120	93
B	78	82	84	90	81
C	66	100	72	86	85
D	107	83	83	87	114
E	73	69	75	78	59
Control			100 (339 lb/ac pure seed)	No S.D. at P = 0.05	
Germination %					
A	80	59	- @	66	64
B	53	74	79	71	66
C	76	67	59	59	60
D	86	78	57	76	72
E	56	60	61	41	54
Control			70 No S.D. at P = 0.05		

@ Dichlorprop not used on first date

Table 4. Experiment 4, 1962 (Timothy S.48)

Spray Date	2,4-D ester	Meco-prop	Dichlor-prop	MCPA/ 2,3,6-TBA	MCPA/ dicamba
Seed yield as % of control					
A	99	104	87	90	85*
B	82*	86	94	75**	80*
C	86	77**	76**	70**	48**
D	48**	72**	70**	26**	15**
E	68**	77**	78**	47**	58**
Control			100 (696 lb/ac clean seed)		
			S.D. (P = 0.05)* = 15%; (P = 0.01)** = 20%		
Germination %					
A	89	88	89	87	84
B	90	90	90	87	84
C	88	93	91	85	74
D	76	84	89	53**	45**
E	89	90	91	85	89
Control			89		
			S.D. (P = 0.05)* = 23%; (P = 0.01)** = 31%		

DISCUSSION

1. While the "control" herbicide 2,4-D was used at the same specially high rate as by Jeater (1955), the other four herbicides were at rates customarily considered suitable for cereals, and it is clear that more often than not they reduced yield and often germination also. All crops were reasonably weed-free and did not in fact require spraying, so that the possible benefit of spraying in removing competition was not measured. Nevertheless, it is clear that in reasonably clean crops, the prevention of seeding by important broad-leaved weeds and the consequent avoidance of cleaning losses would be unlikely to offset the loss of yield due to spraying, not to mention its cost.

2. The majority of significant effects were from spraying from ear emergence onwards. This supports the current recommendation. In experiment 4, however, there were significant losses from spraying timothy 3 or 5 weeks before emergence, in the "safe period", while no experiment gave evidence of higher yield levels in this period. Indeed, experiment 3 and 4 on the whole suggest that at least within the 10-week period before ear emergence the earlier spraying was done the better.

3. Although yield losses occurred, no morphological abnormalities were observed comparable with those reported by Jeater. Observation was of course much less detailed, and single plants might show effects which were hidden or suppressed in a normal crop. Moreover, Jeater's method (1955) of spraying plants individually within a screen might produce effects not caused by normal spraying.

4. There is a suggestion in some of the results that the two MCPA mixtures may have more profound effects than the two phenoxypropionics; for example in the reduced germinations at dates D and E, and in the yields at dates A and B in experiment 4.

5. The lack of significance in the fescue results compared with the timothy at first sight suggests that there may be important species or variety differences, or even that S.48 timothy is specially susceptible. In a standing crop of timothy, however, it is easier to harvest accurately than in lodged fescue, so that there is probably less discrepancy between intended and actual cut areas. The fescue results support all the general conclusions, although less forcibly than the timothy results.

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HERBICIDES IN GRASS SEED PRODUCTION

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Summary Methods of effectively establishing grass seed crops at low rates of seeding have been investigated. An experiment with PCP on S.215 meadow fescue indicated that considerable success can be achieved by using pre-emergence chemical sprays on direct-sown crops. Weeds are effectively controlled and there is better growth of the grass plant following their use, giving promise of increased seed yields. In other experiments, not described in detail, paraquat has been applied to mature drilled stands of various varieties to control weeds and seedlings from shed seed in the inter-row spaces, and also to attempt to control tiller density within rows.

INTRODUCTION

The greater proportion of the herbage seed crop in England and Wales are established under a cover crop, but quite a large acreage is sown direct. Peto (1962) gives the following percentages of the acreage in the latter category, namely, perennial ryegrass 8.8; cocksfoot 37.7; timothy 26.4, and meadow fescue 22.0. Crops thus sown frequently have to contend with severe weed infestation, the control of which by mechanical cultivation when grown in drills adds greatly to the total cost of the seed produced. Earlier studies (Charles and Lewis 1963) indicated the possibilities of using pre-emergence chemical sprays to combat this problem.

The experiment described below, tested the effect of pre-emergence applications of PCP on S.215 meadow fescue, in terms of weed control and crop establishment.

METHOD AND MATERIALS

Seed of S.215 meadow fescue was sown at 1, 2, 3 and 4 lb/ac in a split plot design of four replicates on a seed bed prepared two weeks in advance and subjected to single applicationsof PCP with wetting agent at 3 lb in 60 gal water/ac immediately prior to sowing and six days later before the grass seedlings emerged. A third of the experimental area was left without herbicide treatment as the control.

The grass seed was sown in rows 18 in apart on 11 June 1964 and data were collected on the growth of herbage by estimating the number of plants/ft of drill, and also the ground cover by eye estimation on a scale of 0 = no cover, 10 = full cover, from each sub-plot. Twenty plants per treatment were lifted and weighed after drying and the number of tillers per plant counted.

Pre- and post-treatment weed estimates were made of ground cover on a percentage basis; the dry weight estimates were calculated from two quadrats of 2 ft x 2 ft sampled from each sub-plot.

RESULTS

The area was well covered with arable weeds by the time of sowing. Stellaria media and Polygonum persicaria were the most common on the basis of ground cover estimates, but Sinapis arvensis and Polygonum persicaria were the most robust and were the highest contributors to dry weight bulk (Table 1).

Table 1

Weed population at sowing

	Per cent	
	Dry wt	Ground cover
Sinapis arvensis	20.7	7.3
Polygonum persicaria	20.3	19.0
P. aviculare	12.7	11.6
Convolvulus arvensis	17.9	6.4
Stellaria media	10.0	33.2
Miscellaneous spp.*	18.4	22.5

*Agropyron repens, Chenopodium album, Spergula arvensis, Fumaria officinalis, Poa spp.

The response to PCP was immediate after both sprayings and all the weeds were badly affected within 24 hours; the plots in the pre-sowing treatment soon gave the impression of having been freshly prepared. There was a slight recovery in Polygonum persicaria and P. aviculare in the area which had the second spraying and some plants of Agropyron repens soon began to put out fresh shoots.

The treated plots continued to show the benefit of the herbicide even after the lapse of two months although there was a fresh ingress of weeds. Table 2 indicates the effect of treatment on the growth of the crop grass.

Table 2

Growth and establishment of S.215 meadow fescue

Application of PCP	Seed rate lb/ac	Ht of plant to tip of longest leaf (in)	Density scale 0-10	Plants/ft length of row	Dry wt (gm) per 100 plants
11.6.64	1	5.25	3.75	2.25	25.1
	2	6.00	6.25	5.00	15.3
	3	6.00	7.25	6.00	23.6
	4	7.75	9.25	10.00	15.4
17.6.64	1	3.00	2.00	1.50	9.3
	2	4.00	4.25	4.00	15.2
	3	4.75	6.50	5.25	28.5
	4	6.00	7.75	7.75	11.0
Control	1	1.75	1.00	0.75	2.3
	2	2.00	2.75	2.25	6.3
	3	2.25	4.00	4.75	14.5
	4	2.50	7.25	8.00	7.6

Establishment was very poor on the control plots; the plants appeared stunted and did not tiller vigorously, and from the seed production aspect would have been regarded as valueless. The treated plots, on the other hand, indicated that the best results taking plant density and weight into consideration were obtained from the 3-4 lb/ac seeding. The plants had grown well and the second ingress of weeds did not seem to affect them adversely.

The data on individual plants show that pre-emergence treatment benefits the plant by allowing more scope for tillering and growth as is shown in Table 3.

Table 3

Growth of S.215 meadow fescue in relation to weed cover: mean for all seed rates

Application of PCP	Per cent weed cover after 2 months	Tillers/plant	Wt (gm)/100 plants	Wt (gm)/100 tillers
11.6.64	17.5	2.30	19.9	8.68
17.6.64	45.0	2.40	16.0	6.70
Control	70.0	1.75	7.7	4.42

The fact that tillers are more robust in a comparatively weed-free environment is important since these will produce good seed heads and the resultant crop will be heavier and more uniform than that from plants with a preponderance of weak tillers.

DISCUSSION

Young seed crops

In addition to the experiment described, other experience in using pre-emergence sprays (either PCP at 3 lb/ac or paraquat with a wetting agent at 4 lb/ac) on seed beds prepared one or two weeks before sowing has been highly successful in establishing grass and clover seed stands for investigational purposes. Even better control has been obtained when this was followed within one month, at a vulnerable stage of the young weed seedlings, by a spray of MCPA - at reduced strength if necessary for susceptible species such as timothy. This method has given adequate control up to the first harvest.

Weed control in established drilled stands

Very often there is a fresh ingress of weeds after the first harvest. Such species as _Holcus lanatus_, _Bromus mollis_, _Alopecurus myosuroides_, _Plantago major_ and _Matricaria inodora_ rapidly establish themselves and form a potential source of loss to the herbage seed grower because of the difficulty of removing their seeds from the harvested crop. There is also another source of trouble. Seedlings from shed seeds establish themselves and are a source of genetic impurity especially in the early stages of varietal multiplication. Mechanical cultivation can control these weeds but this tends to encourage further infestation by bringing seeds to the surface, and it also makes harvesting more difficult on account of the ridged effect produced in drilled stands. Effective control is possible by using the newer herbicides and excellent results have been obtained with paraquat applied at 2-4 lb/ac in the inter-row spaces with jets adequately guarded. Experience with this method of paraquat application has shown that care is of paramount importance since drift damage can very readily occur. A drip delivery spraying mechanism, however, will do much to eliminate this trouble. By this paraquat treatment it has been possible to maintain a seed stand of meadow fescue for two harvest years without any diminution of yield and without mechanical cultivation. The fact that spraying operations can often be done when mechanical cultivation would be impossible is of considerable value to the seed grower and in certain crops allows greater flexibility in methods of sowing, for example, timothy which now can only be sown broadcast on heavy land, could then be grown in wide drills.

Herbicides for optimum tiller production

In seed production the aim is to manage the crop to produce the maximum number of seed-producing tillers. Langer and Lambert (1959), Wilson (1959), Ryle (1963) and Lewis (1962) have shown the importance of stimulating the production of early-formed tillers in the seed crop to be harvested the following year. Certain chemicals e.g. dalapon tend to have some selective influence when used in low concentrations (Charles and Lewis 1963; Jones 1962), and it might be possible therefore to a limited extent to control the pattern of tiller behaviour of the herbage seed crop. A preliminary trial at Aberystwyth using paraquat at various concentrations from 2-16 oz/ac on a seed stand of red fescue as an overall spray proved inconclusive, but the fact that the delineation of the treated and untreated plots was very distinct pointed to the possibility of chemical gapping of continuous rows as a means of producing the ideal tiller density. Lambert (1963) has already shown that mechanical gapping of drill stands of S.37 cocksfoot seed crops improved seed yields by as much as one-third over a three-year period. Further work is being done at the Welsh Plant Breeding Station on using herbicides for gapping.

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WEED CONTROL IN LUCERNE AND RED CLOVER SEED CROPS

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Summary In 1963 the production of certified French seeds amounted to 11,000 qx for lucerne and 3,250 qx for red clover.

At present the products used for weed control on young lucerne crops are : neburon, dinoseb, 2,4-DB. After the first year of the crop, it is possible to apply diuron in the winter. Dinoseb and MCPB are used for weed control in red clover.

In trials carried out in 1963-64, pre-emergence dacthal, post-emergence DP 634, ioxynil and bromoxynil seem to be selective for lucerne. Dichlobenil, dichlorothiobenzamide and bromacil are promising for weed control in lucerne and their selectivity should be confirmed.

Pre-emergence spraying of red clover with dacthal seems to be interesting. The study of dichlobenil and dichlorothiobenzamide is to be carried on. Paraquat is very selective in red clover.

INTRODUCTION

The output of certified seeds of lucerne and red clover in France are given in table 1.

Table 1
French production of lucerne and red clover seeds.

	Year	Crops Surface (in ha)	Gathered Quantities (in qx)	Average Yield (in qx/ha)
Lucerne	1962	3,300	7,950	2·4
	1963	6,900	11,000	1·6
Red Clover	1962	1,000	982	0·98
	1963	2,180	3,250	1·5

Besides, an important but undetermined amount of non-certified seeds comes from crops grown mainly for fodder.

Seed crops area is much more important for lucerne than for red clover; it is increasing rapidly because of the rise of the seeds price. These crops are generally sown in lines 40 to 80 cm apart. They are easily infested by weeds. These can prevent a regular emergence of the crop. Moreover, strongly infested crops cannot be certified for seed.

The following chemicals are presently used :

- Neburon, at 2 to 3 kg/ha when sowing lucerne. It is very toxic for red clover.
- Dinoseb is used in lucerne and clover crops from the two trifoliate leaf

stage onward.

- 2,4-DB, at a dosage of 2·5 to 3 kg/ha, is used on lucerne at the 2 to 6 trifoliate leaf stage.

- MCPB is used on red clover under the same conditions.

- Diuron is used when no vegetation of lucerne occurs in the winter, at 2 to 3 kg/ha, provided the crop has been grown for more than one year. It is particularly used for the destruction of weed grasses.

These different chemicals are generally sufficiently active against young weeds, but control of perennial plants remains an important problem. The experiments carried out in 1963-64 were aimed at finding better products than those used up to now for weed control at the time of establishment of the crop and during the following years.

MATERIALS AND METHODS

Experimental products

1° For weed control during the year of establishment

	Experimental doses (in kg/ha)		
- monolinuron	0·75	1·5	3
- linuron	1	2	4
- chloroxuron	2	4	8
- buturon	0·75	1·5	3
- eturon	0·5	1	2
- neburon	1·25	2·5	5
- bromacil	0·5	1	2
- 3 cyclo hexyl 5-6 trimethylene uracile ("DP 634")	1	2	4
- paraquat (without wetting agent)	0·4	0·8	1·6
- 2,4 dichlorophenyl-4-nitrophenylether ("FW 925")	1·25	2·5	5
- dimethyltetrachloroterephtalate ("Dacthal")	1·5	3	6
- bromoxynil	0·25	0·5	1
- ioxynil	0·2	0·4	0·8
- phenylcarbamoyloxy-2 N ethylpropionamide ("RP 11 561")	1	2	4
- dichlobenil	1·5	3	6
- 2,6 dichlorothiobenzamide	1	2	4

2° For weed control after the first year of the crop

	Experimental doses (in kg/ha)					
	Lucerne			Red Clover		
- diuron	2	4	8	1	2	4
- monuron	2	4	8	1	2	4
- linuron	2	4	8	1	2	4
- bromacil	0·5	1	2	0·25	0·5	1
- isocil	0·5	1	2	0·25	0·5	1
- simazine	1	2	4	0·5	1	2

	Experimental doses (in kg/ha)					
	Lucerne			Red Clover		
- atrazine	1	2	4	0·5	1	2
- prometryne	1·5	3	6	0·75	1·5	3
- simetryne	1·5	3	6	0·75	1·5	3
- 2 methylthio-4 isopropylamino-6 (γ methoxy propylamino)-s-triazine (G 36 393)	1	2	4	0·5	1	2
- methylthio-4 ethylamino-6 (γ methoxypropylamino)-s-triazine (GS 12 344)	1	2	4	0·5	1	2
- paraquat (without wetting agent)	0·4	0·8	1·2	0·2	0·4	0·6
- dichlobenil	1·5	3	6	0·75	1·5	3
- 2,6 dichlorothiobenzamide	1	2	4	0·5	1	2

Conditions under which the trials have been made

1° Trials made during the first year of the crop (table 2)

They were aimed at confirming the activity of the different chemicals on weeds and at comparing their toxicity against lucerne and red clover. In these trials, no yield assessments were realised. Only the activity of the herbicides on cultivated plants and weeds was visually scored.

Table 2

Pre-emergence application - Conditions under which the trials have been made during the year of establishment for lucerne and red clover.

N°	PLACES	Soil type	Variety	Sowing (1964)	Treatments 1st Date (1964)	2nd Date (1964)	No. of leaves
	LUCERNES						
1	Cormes (Sarthe)	Calcareous clay	Flamande	6/4	7/4	23/5	5
2	Villeromain (L & C)	Calcareous clay	Flamande	10/4	14/4	21/5	4
3	Chevrières (Oise)	Loamy sand	Flamande	14/4	15/4	22/5	4
4	Gourville (S & O)	Calcareous clay-stony	Gamma	27/4	28/4	28/5	4
5	Crest (Drôme)	Silt	Oméga	13/4	16/4	19/5	4
6	Castelnaudary (Aude)	Calcareous clay		14/4	20/4	13/5	2
	CLOVERS						
7	Courbouzon (L & C)	Loamy sand	Violetta	14/4	14/4	21/5	3
8	Moulon (S & O)	Silt	Goliath	14/4	16/4	24/5	4

2° Trials laid out after the first year of the crop

- triazines, ureas-uraciles :

These trials were carried out in the same way as those made in the first year of the crop. Table 3 shows the experimental conditions. Besides, diuron was used in lucerne yield trials for seed and fodder, in order to determine the maximum dosage of product tolerated by the crop. The conditions

Table 3

Conditions under which the trials have been made after the first year of the crop.

N°	PLACES	Soil type	Variety	Sowing	1st Treat. Date	1st Treat. Stage	2nd Treat. Date 1964	2nd Treat. height of lucerne
	LUCERNES							
11	Seine & Oise	Silt	Flamande	April 62	5/12/63	(1)	28/3	5 cm
12	Seine & Oise	Calcareous clay	Gamma	"	6/12/63	(1)	26/3	5 cm
13	Eure & Loir	Calcareous clay stony	Gamma	"	7/12/63	(1)	3/4	10 cm
14	Loir & Cher	Silt clay	Flamande	"	11/12/63	(1)	10/4	15 cm
15	Oise	Silt	Flamande	"	20/ 2/64	(1)	27/3	5 cm
16	Tarn	Silt clay		April	27/ 2/64	(2)		
17	Aude	Calcareous clay	du Puits	63	28/ 3/64	(2)		
18	B.-Alpes	Calcareous clay		"	10/4/ 64	(2)		
	CLOVERS							
19	Loir & Cher	Loamy sand	Violetta	April 63	11/12/63	(1)	10/4	10 cm
20	Sarthe	Calcareous clay	Goliath	April 62	9/1/64	(1)		
21	Oise	Calcareous clay		April 63	24/ 2/64	(1)	27/3	5 cm

(1) Dormant
(2) Commencement of spring growth

of realisation of these trials are shown in table 4.

- paraquat :

On one hand, in 5 experiments, visual scores were made to determine the action of the chemical on weeds and on the crop. On the other hand, in the same 5 trials, yield measurements of paraquat-sprayed lucerne were made for seed and fodder.

RESULTS

Weed control during the first year of the crop

It was impossible to estimate the activity of the various chemicals on the young grass weeds because the latter were too scarce.

1° Ureas and uraciles

Bromacil, monolinuron, linuron, chloroxuron, "DP 634", neburon, were very effective on weeds when applied before their emergence even at the lowest dosage used. They controlled : Chenopodium album, Sinapis arvensis, Matricaria chamomilla, Rumex acetosa.

The following weeds were less susceptible : Sonchus arvensis, Polygonum aviculare, Amarantus retroflexus. Mercurialis annua proved to be very resistant. Buturon was at the dosages used, slightly less effective than the other weed killers. Eturon gave only poor control. Finally, neburon was sometimes quite unsatisfactory when the ground was dry at sowing time. Applied after the emergence of the weeds, monolinuron, chloroxuron and buturon still gave good control.

Table 4

Conditions under which trials have been made with diuron.

N°	PLACES	Soil Type	Sowing	Variety	Treatment Date	Crops N°	Crops Date	Crops Stage
	Fodder							
D1	Sauveterre St Denis (T & G)	Silt	Oct 61	Socheville	29/11/63	1	15/5/64	Budding
						2	6/7/64	Start of flowering
						3	11/8/64	Flowering
D2	Sauveterre St Denis	Silt	April 63	Socheville	29/11/63	1	12/5/64	Budding
D3	Orphin (S & O)	Calcareous clay	April 62	Flamande	10/12/63	2	1/7/64	Budding
						3	12/8/64	Flowering
D4	Cormes (Sarthe)	Loamy sand	April 62	Gamma	4/1/64	1	8/6/64	Flowering
						2	7/8/64	End of flowering
	Seed							
D5	Massac (Tarn)	Calcareous clay	26/3/63	Flamande	18/12/63			Seed maturity
D6	Sauveterre St Denis	Silt	April 63	Socheville	29/11/63			Seed maturity

Table 5

Conditions under which trials of yield have been made with paraquat.

N°	PLACES	Soil type	Sowing	Variety	Treatments Date	Treatments Stage	Crops N°	Crops Date	Crops Stage
	Fodder								
P1	Cormes (Sarthe)	Sandy loam	April 63	Gamma	6/4/64	10 cm	1	8/6/64	Flowering
							2	6/8/64	End of flowering
P2	Francière (Oise)	Calcareous clay	April 62	Flamande	26/5/64	(a)	1	20/7/64	Flowering
							2	7/9/64	Flowering
	Seed								
P3	Tarn	Calcareous clay	April 63	Socheville	28/2/64	4 cm			Maturity
P4	Massac (Tarn)	Calcareous clay	April 63	Socheville	15/3/64	8 cm			Maturity
P5	St Christophe (C-M)	Calcareous clay	April 63	Socheville	21/5/64	(b)			Maturity

(a) 5 days after mowing   (b) 6 days after mowing.

In pre-emergence, no urea or uracile proved to be, at the dosages used, sufficiently effective to be used on lucerne, except neburon and eturon ; the latter was too poor a weed-killer for its use to be considered.

In post-emergence, chloroxuron and buturon were less detrimental and did not affect lucerne at the lowest dosages used. DP 634 proved to be the most selective, as it did not injure lucerne at the dosage of 2 kg/ha.

Red clover was more sensitive to ureas and uraciles than lucerne. All these products depressed red clover, even at low dosages. The use of these chemicals cannot be considered.

2° Other herbicides

In pre-emergence dichlobenil and dichlorothiobenzamide both had an effective and similar activity on weeds. Chenopodium album, Sinapis arvensis, Matricaria chamomilla, Sonchus arvensis, Rumex acetosa, Polygonum aviculare, were destroyed even at 1·5 kg/ha of dichlobenil and 1 kg/ha of dichlorothiobenzamide. Dacthal was effective even at the minimum dosage of 3 kg/ha against a number of weeds : Chenopodium album, Solanum nigrum, Rumex acetosa, Polygonum aviculare. But it did not give any control of : Sinapis arvensis, Amarantus retroflexus, Sonchus arvensis. "FW 925" did not generally affect the weeds, except Polygonum aviculare. Ioxynil, bromoxynil, paraquat and RP 11 561 were not studied in pre-emergence.

In post-emergence of weeds, dichlobenil and dichlorothiobenzamide had a herbicidal activity similar to pre-emergence. Dacthal, a pre-emergence weed-killer, was not effective. "FW 925" (2·5 and 5 kg/ha) gave good control of weeds such as : Chenopodium album, Sinapis arvensis and Solanum nigrum. Lacking persistence, paraquat did not prevent at all the development of weeds. Ioxynil and bromoxynil were good weed-killers, event at 0·25 kg/ha. RP 11 561 (1 and 2 kg/ha) was active only against Chenopodium album and Sinapis arvensis.

Dichlobenil and dichlorothiobenzamide destroyed lucerne, in pre-emergence, at every dosage. In post-emergence, dosages of 3 kg/ha dichlobenil and of 2 kg/ha dichlorothiobenzamide had no visible toxicity on lucerne. Dacthal was well tolerated by lucerne, even at 6 kg/ha. FW 925 was very toxic in pre-emergence, but well tolerated up to 2·5 kg/ha in post-emergence. Paraquat killed most of the young lucernes, even at 0·4 kg/ha. Ioxynil (0·8 kg/ha), bromoxynil (1 kg/ha) and RP 11 561 (4 kg/ha) did not seem to injure lucerne, even at 1 kg/ha. Post-emergence treatments were carried out when lucerne had 4 or 5 trifoliate leaves, and it cannot be excluded that at an earlier stage the crop can be less resistant.

Dichlobenil and dichlorothiobenzamide had the same action on red clover as on lucerne. In red clover post-emergence dichlobenil at 3 kg/ha and dichlorothiobenzamide at 2 kg/ha were rather well tolerated. The crop always resisted well to dacthal. FW 925 (5 kg/ha) was very toxic in pre-emergence but well tolerated in post-emergence. Paraquat (0·2 kg/ha) seriously injured young red clover when sprayed at the 3-4 leaf stage. Ioxynil and bromoxynil were better tolerated by lucerne than by red clover ; but dosages above 0·2 kg/ha injured both crops. Post-emergence RP 11 561 (4 kg/ha) had no action on red clover.

Weed control after the first year of the crop

1° Triazines

Triazines were not very active against broad-leaved plants. Atrazine (1 kg/ha) was more effective than simazine, particularly against Plantago sp. Prometryne was irregular in activity from one trial to another. Triazines

G 36 393, GS 12 344 and simetryne did not give good weed control, even at high dosages against weed grasses : Agrostis stolonifera, Alopecurus myosuroïdes, Poa pratensis. Simazine and atrazine had very good results, even at 1 kg/ha.

Lucerne was depressed by atrazine, even at the lowest dosage (1 kg/ha). Simazine, better tolerated, was nervertheless toxic at 2 kg/ha. Lucerne resisted fairly well to prometryne (6 kg/ha), symetryne (6 kg/ha), G 36 393 (4 kg/ha), GS12 344 (4 kg/ha).

Simazine and particularly atrazine injured red clover even at the lowest dosage (0·5 kg/ha). Red clover withstood prometryne (1·5 kg/ha), simetryne (1·5 kg/ha), G 36 393 (1 kg/ha), GS 12 344 (1 kg/ha) but these dosages often did not provide satisfactory weed control.

2° Ureas and uraciles

Diuron, monuron, and linuron often were not effective enough against broad-leaved weeds particularly against Taraxacum, Plantago and Ranunculus. Isocil (1 kg/ha) and bromacil (1 kg/ha) appeared to be more effective against these plants. These products were all very active against weed grasses, even at the lowest dosages, except linuron which did not control deep-rooted grasses adequately.

Spring applications of ureas and uraciles at the start of lucerne vegetation, were always harmful. When the treatments were carried out in the winter, when there was no growth, monuron was little selective. Isocil and bromacil did not injure lucerne at 0·5 and 1 kg/ha ; they became rather toxic from the 1·5 kg/ha upwards. Linuron and diuron appeared to be well tolerated at 2 and 4 kg/ha.

The yield trials in table 6 show that even at only 3 kg/ha of diuron, the first production of fodder after the treatment decreased sometimes, but low dosages of diuron did not impair significantly the production of seeds, the latter being affected only at 6 kg/ha of diuron.

Linuron injured red clover less than the other ureas and uraciles. However, the dosage tolerated by the crop (2 kg/ha) was but little active against a number of weeds, particularly against deep-rooted grasses.

3° Paraquat

Paraquat activity was poor on deep-rooted broad-leaved plants which usually infest lucerne and red clover crops. It did not seem to be appreciably better than mowing. Conversely this chemical very effectively controlled annual weed grasses, such as : Lolium italicum, Agrostis spicaventi, Alopecurus myosuroïdes, even at the lowest dosage (0·8 kg/ha).

The results in table 7 show that paraquat injured lucerne when the treatments were carried out on young shoots, at the beginning of the vegetative cycle or after a cut, event when the young shoots were very small. Yet, it seems that the depression caused by paraquat on lucerne vegetation did not reduce the seed production. The treatments made immediately after mowing were apparently well tolerated by lucerne at 0·8 kg/ha.

Spring applications of paraquat destroyed the foliage of red clover, but the crop started again very vigorously. Red clover seems to be more resistant to paraquat than lucerne.

4° Dichlobenil and dichlorothiobenzamide

Spring treatments with both chemicals had an interesting activity on a number of weeds, such as Plantago sp. and Taraxacum dens leonïs. They

Table 6

Yield of lucerne after spraying with diuron (1)

Trials \ Doses	0	2	3	4	6	L.S.D.	Coeff. of var.	Control yield (2)
Fodder								
D1 - 1st mowing	100	91.2	91.1	79.8	54.7	8.1	7.9	5.4
2nd mowing	100	108.3	99.3	96.1	82.2	10.7	8.2	5.1
3th mowing	100	78.9	76.6	72.7	76.3	NS	23.3	0.7
D2 - 1st mowing	100	91.1	82.9	81.6	60.0	10.9	10.9	4.9
D3 - 2nd mowing	100	90.4	86.3	80.0	54.7	9.2	8.1	4.5
3th mowing	100	104.3	118.3	93.6	82.0	NS	13.2	3
D4 - 1st mowing	100	92.7	105.2	88.0	76.8	17	13.3	7.5
2nd mowing	100	89.9	98.7	99.7	95.7	6.3	4.7	3.1
Seed								
D5 - Massac	100	98.9	96.6	96.0	85.5	8.5	6.1	6.1
D6 - Lot & Garonne	100	107.8	105.3	100.2	110.7	NS	17.4	3.2

Table 7

Yield of lucerne after spraying with paraquat (1)

Trials \ Doses	0	2	4	6	8	L.S.D.	Coeff. of var.	Control yield (2)
Fodder								
P1 - 1st mowing	100	77.7	62.2	60.3	53.9	10.4	12.2	6.5
P2 - 1st mowing	100	86.3	85.2	83.0	79.7	9.5	7.8	1.7
2nd mowing	100	86.1	91.3	90.7	99.8	NS	11.6	4
Seed								
P3	100	94.5	102.4	96.6	98.2	NS	14.8	4.5
P4	100	106.5	94.3	88.1	96.1	NS	12.8	3.9
P5	100	91.0	91.4	86.1	81.1	11.6	9.3	2.9

(1) Underlined results are different from those of the untreated control plot at the level of significance - P = 0.05.

(2) For fodder, in t/ha of dry matter.
For seed, in seeds qx/ha.

acted more poorly on weed grasses.

At higher dosages, such as 4 kg/ha dichlobenil and 6 kg/ha dichlorothiobenzamide, lucerne was set back, but this reaction disappeared during the vegetation.

Both herbicides had a similar activity in red clover crops. Dosages of 3 kg/ha dichlobenil and 2 kg/ha dichlorothiobenzamide did not visibly injure clover.

DISCUSSION

Weed control during the first year of the crop

1° Lucerne weed control

Among the tested ureas and uraciles, DP 634 seems to be the most promising. Pre-emergence dacthal proved to be very selective. Selectivity of buturon, chloroxuron, ioxynil, bromoxynil, dichlobenil, dichlorothiobenzamide and FW 925 justifies further investigation.

2° Red clover weed control

The tested ureas, uraciles, ioxynil and bromoxynil are too toxic. On the contrary dacthal appears to be very selective in pre-emergence. Dichlobenil and dichlorothiobenzamide should equally be studied more thoroughly.

Weed control after the first year of the crop

1° Lucerne weed control

Diuron can be used in the winter, if the crop is more than a year old, at 2 to 3 kg/ha. Yet, it is necessary to take the strength of the crop into account. Deeply rooted lucerne is more resistant to diuron than when superficially rooted. Bromacil also is promising as shown by its herbicidal activity. Triazines generally lack selectivity. Paraquat is of little interest in seed-lucerne crops, because it cannot be used in crops in full growth and because seed is, as far as possible, harvested without a previous fodder cut. Dichlobenil and dichlorothiobenzamide seem to be rather well tolerated by lucerne.

2° Red clover weed control

The triazines, ureas and uraciles tried are of limited interest, but the use of paraquat can be considered. Finally, dichlobenil and dichlorothiobenzamide seem to be well tolerated by red clover, but the weed control problem for that crop remains unanswered.

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Research Summary

PRELIMINARY EXPERIMENTS WITH IOXYNIL AND BROMOXYNIL IN FORAGE LEGUMES.

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Summary Preliminary field experiments have shown that bromoxynil is safer than ioxynil on lucerne, red and white clover. At a dose of 8 oz/ac, which gave good control of the major weeds present, bromoxynil had no adverse effect on the stand of lucerne, and yields were substantially increased over those of the weedy controls. Red and white clover were more sensitive than lucerne to both compounds.

INTRODUCTION.

The possibility of obtaining selective weed control in forage legumes with ioxynil (4-hydroxy-3,5-diiodobenzonitrile) and bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) was first suggested by Carpenter and Heywood (1963). In greenhouse experiments, Holly and Holroyd (1963) found that ioxynil at 8 oz caused very little damage to lucerne sprayed at the 2-4 leaf stage. Damage was more severe to both red and white clover, but not so great as to preclude the possibility of recovery under field conditions. A field screening experiment carried out in 1963 confirmed that lucerne was more tolerant than either red or white clover, and also indicated that bromoxynil caused less damage than ioxynil to all three crops (Carpenter et al 1964).

Further field experiments were therefore carried out during 1964 to determine the tolerance, under field conditions, of lucerne, red and white clover to these compounds. This paper summarises these results, which will be more fully reported in due course.

METHODS AND MATERIALS.

Ioxynil (sodium salt) and bromoxynil (potassium salt) were applied, without wetter, at doses of 2, 4, and 8 oz/ac to both direct and undersown crops of lucerne (var. Socheville), red clover (var. English broad red) and white clover (var. S 100). MCPA salt, at the same doses, was used as a standard. The cover crop was wheat (var. Jufy). Each treatment was applied, in 15.7 gal/ac, at two growth stages of the legumes, namely at the spade (primary) leaf and 2-3 trifoliate leaf stages. Plot size was 6 x 12 ft. with two replications in the red and white clover experiments and three replications in the lucerne experiment.

The effect of treatments on the crops was assessed by leaf counts in 1 x 1 ft^2 quadrat per plot (4-5 weeks after spraying) and yields of fresh weight from 2 x $\frac{1}{2}$ yd^2 samples per plot (2 months after the second spraying).

Weed control was assessed by counts taken in 2 x $\frac{1}{2}$ yd^2 quadrats per plot.

RESULTS.

Two to three days after spraying, red and white clover were badly scorched by all rates of both ioxynil and bromoxynil, whereas lucerne showed slight scorching on the ioxynil plots only. A fortnight after spraying many of the scorched leaves had completely withered, and, in the case of the very young seedlings present at the first growth stage spraying, the damage was severe enough to cause the death of many plants.

The counts (Table 1) show that reductions in leaf numbers were still apparent four to five weeks after spraying, both red and white clover being severely affected by ioxynil, and red clover by bromoxynil. These reductions were greater when the treatments were applied at the early growth stage, and the direct sown plants (the figures of which have not been included) of lucerne and red clover were damaged more than those undersown. The undersown white clover, however, was more severely damaged than that sown direct, and this was probably due to the warm dry conditions which followed spraying. Recovery of the shallow rooted white clover, when competing for moisture with the cereal cover crop, was much less than that of the deeper rooted red clover and lucerne.

Weed competition restricted crop growth in the unsprayed plots and the fresh weight yields of the treated plots were generally equal to or better than those of the controls (Table 2). Details of the control of the major weeds is shown in Table 3.

TABLE 1. Initial effect of treatments on crops. Leaf counts per ft.^{2} (4-5 weeks after spraying) as % of control.

Crop.	Growth stage at spraying	Ioxynil oz/ac 2	4	8	Bromoxynil oz/ac 2	4	8	MCPA oz/ac 2	4	8
Lucerne	Spade leaf	51	72	54	109	103	109	105	40	42
	2-3 trifoliate	109	89	81	103	145	132	84	33	14
Red clover	2-3 trifoliate	101	61	54	83	85	51	78	101	83
White clover	2-3 trifoliate	121	72	48	123	125	103	131	138	56

TABLE 2. Effect of treatments on crop yields. Fresh weights (2-3 months after spraying) as % of control.

Crop.	Growth stage at spraying.	Ioxynil oz/ac. 2	4	8	Bromoxynil oz/ac 2	4	8	MCPA oz/ac. 2	4	8
Lucerne	Spade leaf	136	138	125	134	184*	220*	163*	122	158*
	2-3 trifoliate	158*	181*	171*	183*	182*	223*	149*	137	97
Red clover	2-3 trifoliate	124	132	165*	128	162*	151*	132	166*	165*
White clover	2-3 trifoliate	86	109	119	122	85	198*	115	151	123

* Significantly greater than the control (P=0.05)

TABLE 3. Control of major weed species - % control of numbers.

Weed & Growth stage at spraying.	Control Nos.per yd^2	Ioxynil oz/ac 2	4	8	Bromoxynil oz/ac 2	4	8	MCPA oz/ac 2	4	8
Veronica persica (Buxbaum's speedwell) 6-8 lvs, 3 in.	33	55	89	88	77	85	70	0	8	32
Polygonum aviculare (knotgrass) 4-6 lvs, 1-3 in.	17	0	6	41	53	50	82	0	0	26
Sonchus asper (annual sowthistle) 3-5 lvs, 1½-3 in.	12	4	8	83	92	87	37	0	0	50
Anagallis arvensis. (scarlet pimpernel) 2-4 lvs, ½-1 in.	13	65	100	92	73	73	88	31	38	58

DISCUSSION.

The results obtained in these experiments support the earlier findings of Carpenter et al (1964) that lucerne is the most tolerant of the three legumes and that bromoxynil is less damaging than ioxynil.

The formulations of ioxynil and bromoxynil used in these experiments contained no wetting agent and although most of the weeds present were adequately controlled by 8 oz when sprayed early (with the exception of the control of knotgrass by ioxynil), it is known that for the control of certain species, e.g., Chenopodium album (fat hen) the addition of wetter is desirable.

Other experimental evidence from this country, and from work carried out by workers in Canada (Cook, 1964) has shown that salt formulations with wetter, and ester formulations, of bromoxynil are well tolerated by lucerne at the doses required for good weed control.

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Research Summary

PLANT COMPETITION AND WEED CONTROL IN MAIZE

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SUMMARY

Results from experiments in which the levels of competition are controlled are being used in an attempt to interpret the effects of weed competition in maize. Relatively short periods (2-4 weeks) of competition during the early growth of the crop may be sufficient to reduce final yield. The results emphasize the need for timely weed control. Data from trials with herbicides applied before crop emergence indicate that the more persistent chemicals (atrazine, simazine, linuron) are preferable to 2,4-D formulations for pre-emergence treatments. Discrepancies in the data from the three years of trials seem to be related to differences in water supply. Adequate rainfall in the early stages of crop and weed development is beneficial to herbicidal action (not only of such slightly soluble compounds as simazine but also of the 2,4-D formulations) and also makes maize better able to withstand weed competition.

Provided that bird damage to seedlings is sufficiently contained to permit a final crop density of around 9 plants/yd^2 the average yield of commercial maize crops in S. England should exceed 4 tons/acre dry material. Lower productivity is generally caused by inadequate water supply and this, in turn, usually results from the failure to conserve water for the crop by eradicating weeds.

INTRODUCTION

Evidence from field trials and commercial sowings indicates that late April or early May is the most appropriate time to sow maize in central or Southern England. Normally the mean soil temperature at seeding depth (5 cm.) is then 11-13°C., whereas in the main maize growing areas of U.S.A. the crop is not usually sown until the soil temperature is around 15°C., and rising much more rapidly than in late spring in England. Although final yield of grain or of shoot dry matter per acre in southern England and U.S.A. is strictly comparable, the contrasting environmental conditions lead to differences in rate of growth and dry matter accumulation - and these differences are especially marked in the early stages of crop development. The susceptibility of maize to weed infestation is recognised in U.S.A.; it will obviously be increased by the slower initial growth of the crop under English conditions.

This paper describes work (to be reported more fully later) on competition in maize and the effects of herbicides applied before crop emergence. The effects of variation in plant density are relevant to the study of plant competition. Fourteen experiments involving different plant densities have been conducted since 1952. The earlier experiments - which often involved simultaneous consideration of changes in spatial arrangement of the plants (by alterations in row width) and in the nutritional level of the soil - have been reported (Bunting & Willey, 1959), and the general conclusions then formulated have been confirmed in more recent trials. In these plant density studies only one (final) harvest was taken, but in the 1964 experiment involving different plant densities assessments of yield of shoot dry matter were made at frequent intervals during the growing season. Additional information on the influence of varying periods of competition has been obtained in 1962, 1963 and 1964 by thinning from high plant densities to a standard density at regular intervals.

In these experiments competition was provided by extra maize plants and weed competition has been prevented. In three other trials (1960, 1961 and 1962) competition has been provided by natural weed infestation and the crop cleaned initially at different times (and thereafter kept free from weeds).

In the 3 herbicide trials so far completed (1961, 1962 and 1963) particular attention has been paid to applications before crop emergence. By hand weeding one half of each plot, including unsprayed control plots, information has also been obtained on crop tolerance.

RESULTS

Effects of changes in plant density

At any given density between 3 and 30 plants/yd^2 the changes in spatial arrangement of plants brought about by verying the distance between adjacent drill rows from 30-75cm. (12-30in.) have not markedly influenced productivity. In contrast the effects of changes in plant density are usually considerable. Data from 14 trials indicate that average yields of shoot dry matter per acre at 3, 6, 12, 18 and 27 plants/yd^2 will be 68%, 87%, 107%, 110%, and 113% of the yield obtained with 9 plants/yd^2. At high densities, however, there is a risk of serious lodging and, on balance, a density of 9 plants/yd^2 is considered most appropriate for silage maize production in southern England. Considering the data from the density trials in terms of plant competition, and regarding a density of 9 plants/yd^2 as standard, then the introduction of extra competition from an additional 3, 9 or 18 plants/yd^2 (by providing densities of 12, 18 and 27 plants/yd^2 respectively) has, on average, reduced final yield per plant by 20%, 45% or 62% respectively.

When competing plants were removed at different times during the growing season the data suggest that slight competition (represented by an extra 4.5 plants/yd^2) during the month subsequent to emergence does not affect development. With heavier competition (9 plants/yd^2 in 1963. 18 plants/yd^2 in 1962) during this period, however, final yield was significantly depressed and in 1964, when assessments of yield were made at frequent intervals during the growing season, competition from an extra 9 plants/yd^2 measurably reduced yield per plant within 2 weeks of emergence.

Effects of weed competition

When competition to maize grown at 9 plants/yd^2 was provided by natural weed infestation and studies were made of the effects of differing periods of competition, the results, given in the table below, were less precise statistically but generally confirmed the need for early weed control.

The effects of time of weeding on yields of maize

	Yield of Shoot (Tons/ac. dry material)		
Year	1960	1961	1962
Weed-free from emergence	4.94	4.72	3.74
Weeds removed 2 weeks after emergence	4.62		
3 " " "			3.45
4 " " "	4.63	4.51	
6 " " "	4.36	4.45	1.30
Control (unweeded)	3.58	3.70	0.82
Sig diff $p = 0.05$	0.72	0.53	0.47

Weed control

These data on competition suggest that herbicides applied before crop (or weed) emergence are most likely to be satisfactory for maize, and trials were initiated in 1961. Simazine and 2,4D amine were then compared at rates of 0.75, 1.50 or 3.00 lb/ac a.i. Very dry weather prevailed for 5 weeks after sowing and although weed infestation was very light neither herbicide was really effective. On the unsprayed plots the mean density of weeds was only 19/yd^2 (*Chenopodium album* 45% *Polygonum aviculare* 22% *Veronica persica* 19% *P. convolvulus* 4% *Medicago lupulina* 5% of total). *Polygonum aviculare* was not completely controlled; the highest dosage rate of 2,4D amine reduced the number by 60% to only 1.5 pl/yd^2 and simazine removed 85% to leave less than 1 pl/yd^2. but the survivors made such vigorous growth that 20% and 10% respectively of the treated areas were covered and yields of dry material from the maize crop were appreciably lower than on hand weeded plots.

Five herbicides (2,4-D amine, 2,4-D ester, simazine, atrazine, linuron) were studied in 1962. Each was applied before crop emergence at two rates (1.5 and 3.0 lb/ac a.i.). Rainfall in the twe weeks following the application of herbicides was about average (0.84 in. total) but the preceding 3 weeks and the subsequent 5 weeks were very dry. Weed infestation was extremely heavy with average counts of 701/yd^2 on control plots 4 weeks after crop emergence. The main weeds present were *Stellaria media* (nearly 50% of the total number) *Atriplex patula* (27%) *Chenopodium album* (15%) *Veronica persica* (5%) and *Sonchus oleraceous* (3.5%). Neither 2,4-D formulations gave really satisfactory control of weeds but simazine, linuron and atrazine were more effective. Simazine controlled all weeds except *Atriplex patula*; linuron failed to eradicate only *Veronica persica* and with atrazine weed control was virtually absolute. Significant differences were found in the competitive effects of surviving weed species. At the lower rates of application the mean density of *Veronica persica* plants surviving the linuron treatment was exactly the same as that of *Atriplex patula* on plots treated with simazine. The effects of the two weeds on crop yield were markedly different, however, since *Atriplex patula* reduced yield of maize by nearly 40% whereas the reduction occasioned by *Veronica persica* was less than 10%.

Conditions in 1963 - with adequate and well distributed rainfall from crop emergence until harvest - were quite different from those prevailing in the two previous years. The same weed species were present as in 1962 but the numbers were lower (120 weeds/yd^2). 2,4-D amine, applied pre-emergence at 2 lb/ac a.i., gave 80-90% control of all weed species present. Simazine, at 1.5 lb/ac a.i., was also much more effective than in 1961 or 1962 and, like atrazine, suppressed weed growth almost entirely. In each year the experimental design enabled comparisons to be made under weed-free conditions between control (unsprayed) plots and plots treated with herbicides. The data do not suggest that the herbicides used have any direct effect on maize development.

DISCUSSION

Results from experiments in which the levels of competition are controlled are being used in an attempt to interpret the effects of weed competition. In maize relatively short periods (2-4 weeks) of competition during the early growth of the crop may be sufficient to reduce final yield. The data point to the need for timely weed control. The results from weed control trials indicate that the more persistent herbicides (atrazine, simazine, linuron) are preferable to 2,4-D formulations for pre-emergence treatments. Discrepancies in the data from the three years of trials seem to be related to differences in water supply. Adequate rainfall in the early stages of crop and weed development is beneficial to herbicidal action (not only of such slightly soluble compounds as simazine but also of the 2,4-D formulations) and also makes maize better able to withstand weed competition. Laboratory studies show that at the relatively low temperatures normally encountered at Oxford in late spring (15-17°C) the maize seedling is 20 cm. tall and has 4-6 leaves before the dry weight of the plant (including root) exceeds the dry weight of the seed sown. Early seedling growth at low temperatures is seemingly dependent on endosperm reserves; the nutrient status of the soil will thus have little effect and competition in the early stages of crop development will be mainly for water. Again, in late July and August, when the maize crop is growing very rapidly, there is a heavy demand for water and limitations in supply during this period (as in 1959 and 1964) will severely reduce final yield.

Provided that bird damage to seedlings is sufficiently contained to permit a final crop density of around 9 plants/yd^2 the average yield of commercial maize crops in S. England should exceed 4 tons/acre dry material. Lower productivity is generally caused by inadequate water supply and this, in turn, usually results from the failure to conserve water for the crop by eradicating weeds.

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ASPECTS OF WEED COMPETITION IN KALE

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Summary. Some of the relationships between weed density, weed flora, nitrogen application and the yield of kale are demonstrated from data obtained in field experiments. The results suggest that weed floras may vary considerably in their effect on the yield of kale, and that competition from weeds early in growth may severely reduce the ultimate yield of kale. It is suggested that a classification of "weed situations" might prove helpful in predicting whether weed control is necessary or not and the form of such control.

INTRODUCTION

Blackman & Templeman (1938), working with cereals in field experiments, demonstrated that both weed species and weed density affected the loss of yield attributable to weed infestation. The importance of weed species and weed density was also shown by Mann & Barnes (1945, 1950) from pot experiments with barley. Bleasdale (1960) with carrots, Hammerton (1962) with kale, and Welbank (1963) with various crop species have all shown, from pot experiments, that weed species differ considerably in their ability to reduce the yields of crop plants. The duration of weed competition has been shown, by Bleasdale (1959), to affect the yield of vegetable crops in the field. He has also suggested (1960) that increased soil fertility may not alleviate the adverse effects of weed competition on vegetable yields. Some of the experiments of Blackman & Templeman (1938) did however indicate that the application of extra nitrogen fertilizer could increase the yield of a weedy cereal crop to a level similar to that of a clean crop without extra nitrogen.

The control of weeds in any crop is economically justifiable only if the increase in yield attributable to such control exceeds in value the cost of control. (This is not invariably true - there are instances where weed control is justified for reasons other than the effect on yield). There are likely to be many situations of low weed density, or where the major weed species is of low competitive ability to the crop in question (or has no effect on quality or ease of harvesting), in which no form of control, or perhaps none but the simplest and cheapest, is economically justified. It would be extremely useful to be able to distinguish, for each crop, a number of "weed situations" on the basis of weed density, major weed species present, and relative stage of growth of crops and weeds. In the case of kale, with which this paper is concerned, the weed situations might be of 3 main types:- (i) where no form of control is likely to be either necessary or worthwhile; (ii) where a single steerage hoeing is likely to be adequate, provided that this is possible; and (iii) where fairly complete control of weeds is likely to be essential and economically justifiable.

The purpose of this paper is to demonstrate some of the relationships between the yield of kale on the one hand, and weed density, weed species, duration of weed-competition and nitrogen application on the other, that have been obtained in field experiments at Aberystwyth.

METHODS & MATERIALS

Experiment 1 (1961) The treatment, layout and results have been described by Hammerton (1963) and only one particular aspects of the results is considered here. The experiment compared 3 herbicides (EPTC, Endothal/propham and DNOC/TCA) applied pre-sowing. These plots received no cultivation subsequent to sowing. These 6 treatments were compared with unweeded and with clean-weeded controls. There were 5 replicates and plot size was 1/349 ac. Marrowstem kale was sown in 20 in. rows at 4 lb/ac on 23rd May. Fertilizer was applied to all plots at the rate of (units/ac) 130 N, 70 P_2O_5 and 70 K_2O in the seedbed.

Experiment 2 (1962) There were 5 treatments in a latin square, namely:- clean weeding from (i) kale emergence, (ii) kale 4-leaf stage, and (iii) kale 8-leaf stage, until the kale met across the rows (end of July), (iv) steerage hoeing twice (simulated by dutch hoe) and (v) unweeded control. Plot size was 1/650 ac, and fertilizer was applied to the seedbed at a rate equivalent to (units/ac) 110 N, 70 P_2O_5 and 70 K_2O. Marrowstem kale was sown in 20 in. rows with a precision seeder at 2 in. spacing on 25th May.

Experiment 3 (1963) In this experiment there were 21 unreplicated treatments, namely all combinations of (i) 3 levels of weed control (none, clean weeding from kale emergence onward, and clean weeding from kale 4-leaf stage onward) and (ii) 7 levels of nitrogen applied to the seed-bed (0, 25, 50, 75, 100, 125 and 150 units/ac N). Plot size was 1/363 ac and all plots received a basal dressing equivalent to (units/ac) 100 P_2O_5 and 100 K_2O in the seed-bed. Marrowstem kale was precision drilled in 20 in. rows at 2 in. spacing on May 7th.

All experiments were harvested in November and sampled for dry-matter yield estimates. The data presented here include (i) yields of kale dry-matter, (ii) weed density (no./yd^2) from random quadrats and (iii) nitrogen % by Kjeldahl.

RESULTS

Table 1 summarises data on the yield of kale from plots clean-weeded by hand from emergence onwards and from unweeded plots, in the 3 experiments described above, and in an additional experiment (Expt. 4; the 1962 experiment of Hammerton, 1963). The greatest loss of yield due to the presence of weeds (83%) was associated with the highest weed density, but the smallest loss (0%) was not associated with the lowest density.

Table 1

The yields of marrowstem kale (tons/ac dry-matter) from unweeded and clean-weeded plots : results from 4 experiments with different weed floras and densities.

Expt.	Weed Flora	Weed* density (no./yd^2)	Yield Unweeded	Yield Clean Weeded	% yield reduction
1.	Stellaria media L. (Vill.) Chenopodium album L. with Veronica spp. Polygonum spp., Agropyron repens L. (Beauv.) and Fumaria officinalis L.	247 23 days after sowing	1.5	3.5	57
2.	Poa annua L. with C. album, S. media, Polygonum spp.	115 30 days after sowing	3.8	3.8	0
3.	Sinapis arvensis L., Spergula arvensis L., with A. repens, P. aviculare L., Veronica spp. C. album.	453 21 days after sowing	0.7**	4.1**	83
4.	S. media, P. annua with Polygonum spp. and self sown barley.	78 28 days after sowing	3.7	4.1	10

*On unweeded control plots. **Means of yields from plots receiving 100, 125 and 150 units/ac N.

The six herbicide treatments of experiment 1 effectively produced six weed densities without substantial changes in the weed flora. The weed flora (shown in Table 1) consisted mainly of Stellaria media (chickweed) and Chenopodium album (fat hen) with a number of other species. The kale showed some slight phytotoxic damage on certain herbicide plots during the early stages of growth, and there was some adverse effect of herbicides on kale density. The relationship between the yield of kale dry-matter (Y) and weed density in no./yd^2 23 days after sowing (x) is shown in Fig. 1. This figure is based on data from a total of 35 herbicide-treated and unweeded control plots; data from the clean-weeded plots are not comparable. A curvilinear regression gives a significantly better fit to these data than a linear regression, the regression equation being $Y = 3.70 - 0.017x + 0.000033\,x^2$. It is clear from this equation, and from Fig. 1, that a given increase in weed density has a greater effect at low weed densities than at high. For

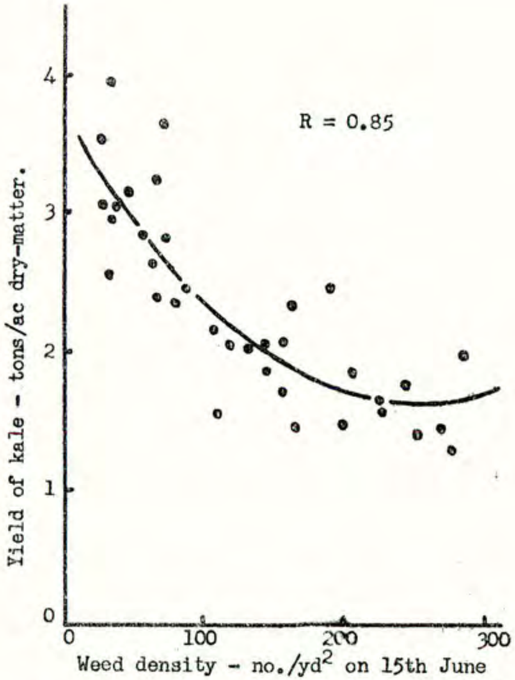

Fig. 1. The relationship between the yield of kale and the weed density on 15th June, 23 days after sowing (Expt. 1).

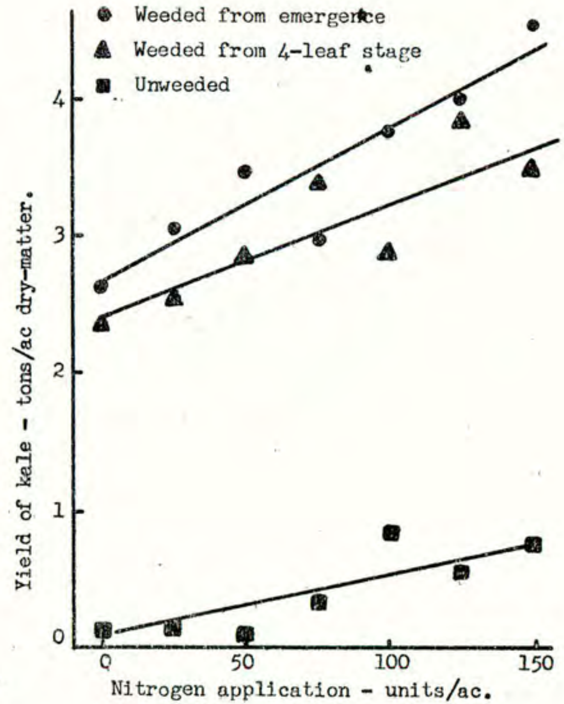

Fig. 2. The effect of time on weeding and level of nitrogen application on the yield of kale (Expt. 3).

example, an increase in density from 50 to 100 weeds/yd^2 reduces the yield by 0.60 ton/ac dry-matter, while an increase in density from 150 to 200 weeds/yd^2 reduces the yield by only 0.25 ton.

The weed flora in experiment 2 was composed mainly of Poa annua (annual meadowgrass) with a lower density of a few dicotyledonous species (see Table 1). The yield of kale was not affected by any of the weeding treatments, as may be seen from Table 2. The weed species present, and the weed density, were clearly such as to offer little competition to the crop. The weeding treatments did however have a significant effect on the % N in the leaf, though neither % N in the stem, nor the N content of the crop were significantly affected. Table 2 shows that clean weeding from the 4-leaf stage and the steerage hoeing treatment both resulted in higher % N in the leaf than the other treatments.

Table 2

Effect of weed control treatments on yield of marrowstem kale (tons/ac dry-matter) and on % N in the kale leaf dry-matter.
Experiment 2, 1962.

Weed control treatment	Yield	% N in leaf
Clean weeded from emergence	3.8	2.98
" " " 4-leaf stage	3.7	3.33
" " " 8-leaf stage	3.9	3.00
Steerage hoed	3.8	3.35
Unweeded control	3.8	2.98
L.S.D. (P= 0.05)	N.S.	0.285

As Table 1 indicates the weed flora of experiment 3 was extremely competitive towards kale. The main components were Sinapis arvensis (Charlock) and Spergula arvensis (spurrey), with couch grass, knotgrass, speedwells and fat hen. Linear regressions have been fitted to the yield data which are shown in Fig. 2. The regression equations of kale yield in tons dry-matter/ac (Y) on level of nitrogen application in units/ac (n) are $Y_1 = 2.66 + 0.011n$ ($\pm$ 0.0021); $Y_2 = 2.41 + 0.008n$ ($\pm$ 0.0025); and $Y_3 = 0.004 + 0.005n$ ($\pm$ 0.0015) where Y_1 is the yield with clean weeding from emergence, Y_2 is the yield with clean weeding from kale 4-leaf stage and Y_3 is the yield without weeding. All the regression coefficients are significant (at P=0.05), but differences between them are not significant, indicating that the response to N application did not differ significantly. Under the conditions of this experiment, a yield of 3 tons/ac dry-matter (equivalent to about 18 tons/ac fresh matter) could be achieved by clean weeding from emergence onward and supplying only 25 units/ac N, or by clean weeding only from the 4-leaf stage and supplying 75 units/ac N. Kale density was rather lower on the control plots than on the two clean weeded treatments, but there were no clear relationships between kale density and N. level.

DISCUSSION

Of the four weed floras summarised in Table 1, two were clearly very competitive towards kale at the density shown, whereas the other two were very much less competitive. In one of the latter cases no weed control at all was necessary (experiment 2), while in the other case steerage hoeing alone might well have sufficed. J. G. Elliott (in personal communications) has reported an experiment in which steerage hoeing a kale crop infested mainly with Polygonum lapathifolium L., proved adequate in terms of yield. On the other hand, where Sinapis arvensis was the main species he found that steerage hoeing alone gave only 60% of the yield of kale from clean weeded plots, and delaying weeding until the kale 4-leaf stage gave only 85% of the yield from clean weeding. The results of experiment 3, and those of Welbank (1963) also suggest that Sinapis arvensis is a serious competitor to kale.

Welbank (1963) found that increase in the nitrogen level did not significantly decrease the effect of weeds on kale, and the data from experiment 3 show that, with a dense and competitive weed flora, nitrogen is no substitute for weed control. In this experiment, delaying weed control until the kale 4-leaf stage resulted in a small loss of yield, as has been found by Elliott (see above). This suggests that a satisfactory residual pre-sowing or pre-emergence herbicide might be preferable to a post-emergence herbicide, particularly if the latter cannot safely be applied until the kale has 4 or more leaves.

A simple relationship between yield and the weed density at an early stage of growth, as found in experiment 1, would clearly be useful in any attempt to predict the need for weed control, if such a relationship is general. A linear relationship between weed density and cereal yield was obtained in some of the experiments of Blackman & Templeman (1938).

With the limited data available, it is clearly impossible to define any "weed situations" in the way suggested in the introduction. However, certain tentative conclusions may be drawn.

(i) Where Sinapis arvensis is the main, or one of the main, weed species, and is abundant, early and thorough weed control is likely to be essential. Similarly, a dense weed flora with main components Stellaria media and C. album is likely to require thorough control. No critical levels of weed density can be stated for these two situations.

(ii) With a sparse weed flora mainly composed of S. media and Poa annua, a single steerage hoeing may be adequate for satisfactory yields. Above a density of approximately $100/yd^2$ four weeks after sowing, more complete weed control may be needed. Polygonum lapathifolium may also be in this category, though no critical levels of density can be given.

(iii) If Poa annua is the major weed, little or no control may be necessary. The presence of over 100 weeds/yd^2 thirty days after sowing (with about ¾ of these P. annua) can apparently be tolerated by kale without loss of yield.

In general, a weed species with a growth habit resembling that of kale (i.e. erect and leafy) is likely to be more competitive (and therefore more likely to require control) than a species with a procumbent habit of growth. The data of Welbank (1963) and of Hammerton (1962) lend some support to this generalisation.

Finally, it should be stressed that the above conclusions are only likely to apply where kale and weeds are at a comparable stage of growth. Kale growing with weeds at a more advanced stage of growth will require thorough weed control to survive and yield well. It is intended to continue these investigations, so that this paper should be regarded as a progress report.

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EVALUATION OF THE TOLERANCE OF KALE TO AMITROLE-T

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Summary Two small plot trials and two farm scale trials involving amitrole-T were laid down in 1963 on differing soil types. Kale sown at different times after the application of amitrole-T appeared to be unaffected with respect to germination, establishment or yield.

INTRODUCTION

Commercial practice has tended to favour kale as a crop to follow a spring application of amitrole-T because as a smother crop it complimented the chemical control. Sandford and Stovell (1960) found that kale was tolerant to amitrole-T residues over a range of soil types. Experiments were laid down to determine the effect on germination, establishment and yield of a standard treatment for control of *Agropyron repens* and *Agrostis spp* (couchgrass) and thus ascertain whether kale could be sown three weeks after a spring application of amitrole-T.

METHODS AND MATERIALS

Four trials were laid down in 1963 in Yorkshire and Nottinghamshire. Two trials (Nos. 1 and 2) comprised small plots (8yd x 6yd) in randomised blocks replicated three times. The other two trials (Nos. 3 and 4) were single ½ ac. plots situated in a field of kale, all operations being carried out by the farmer. For convenience the experimental data for the small plot experiments and the farm scale experiments are given separately. The commercial formulation Weedazol TL which contains 2 lbs 3-amino-1,2,4-triazole and 1.85 lbs ammonium thiocyanate per Imperial gallon was used throughout the series.

Small Plot Experiments (Nos. 1 and 2)

Amitrole-T was sprayed onto soil free of *Agropyron repens* and *Agrostis spp* (couchgrass) at 4 lb/ac (recommended dosage for couchgrass control in arable land) and 8 lb/ac in a total volume of 30 gal. water per acre. Ploughing was done to a depth of 6in. three weeks after spraying. Each plot was given a fertiliser dressing before cultivation equivalent to 4 cwts. per acre of 20-10-10. Cultivations were carried out 3-8 weeks after spraying depending on weather, etc. Untreated control plots were included for each drilling. Spraying was carried out with a Oxford Precision Sprayer, ploughing and cultivations with a Howard 700 cultivator plus implements and the drilling with a Gloster hand seeder unit. At both sites Marrowstem kale was used.

Experiment 1 was situated on a heavy loam soil. Spraying was carried out on 9.Apr.63 and conditions were dry for the three weeks after spraying. The kale was drilled 3, 4, 5 and 7 weeks after spraying.

Experiment 2 was situated on a sandy loam soil. The site was sprayed on 28.Mar.63 with fine warm weather following. Drilling was carried out 4, 5, 7 and 8 weeks after spraying.

Farm Scale Experiments (Nos. 3 and 4)

For No. 3 there was no couchgrass present whereas for No. 4 there was a moderatley heavy infestation. The soil types at the sites were a medium loam and a heavy loam respectively. At both sites amitrole-T was applied at the recommended rate of 4 lb/ac in a total volume of 30 gal. water. Ploughing to a depth of 6-9 in. was carried out after three weeks and then Marrowstem kale was drilled approximately 6 weeks after spraying.

RESULTS

Experiment 1

The establishment of kale plants was scored on a basis 0 = no establishment : 10 = 100% establishment. The first drilling was scored after 4 weeks and the 2nd, 3rd, and 4th drillings after 6 weeks.

The mean establishment scores and yield data are shown in Tables I and II respectively.

TABLE I

Establishment Scores : Experiment I

Treatment	RATE lb ai/ac	Mean Establishment Scores : Weeks between spraying and drilling			
		3	4	5	7
Control-untreated	-	10.0	10.0	10.0	10.0
Amitrole-T	4.0	9.3	10.0	8.7	10.0
"	8.0	8.3	7.0	8.3	10.0

TABLE II
Fresh Weight Yield : Experiment I

Treatment	RATE lb ai/ac	Mean Fresh Wt. of Kale (lb) Weeks between spraying and drilling :			
		3	4	5	7
Control-untreated	-	159.0	161.3	164.0	147.3
Amitrole-T	4.0	186.0	179.3	197.8	155.2
	8.0	159.2	163.0	197.3	139.0

In Experiment 1 occasional kale plants were observed in the plots drilled three weeks after spraying which showed mild forms of stunting, and chlorosis. These effects became less noticeable in later drillings. Indeed poor establishment in the 2nd, 3rd and 4th drilling was more attributable to the difficulty of obtaining a good seed bed due to dry conditions than to the spraying. The kale quickly recovered from these effects and the yield results indicate that no reduction had occurred due to amitrol-T residues.

Experiment 2

The establishment of kale plants was scored on the same basis as in Experiment 1. The mean scores are shown in Table III. No yield data was recorded because the area was affected by a heavy weed infestation and the trial was abandoned.

TABLE III
Establishment Scores : Experiment 2

Treatment	RATE lb ai/ac	Mean Establishment Scores : Weeks between spraying and drilling :			
		4	5	7	8
Control-untreated	-	10.0	10.0	10.0	10.0
Weedazol TL	4.0	10.0	10.0	10.0	10.0
	8.0	10.0	10.0	10.0	10.0

At Experiment 2 no chlorosis or stunting was observed with any of the treatments.

There was no major difference due to the different soil types. On the sandy loam where residues were expected to be higher no effect was noticeable.

Farm Scale Experiments (Nos. 3 and 4)

At both sites no effect due to amitrole-T was observed on the germination or establishment of the kale. In the autumn no difference could be observed between treated and untreated areas with respect to height, density and estimated yield.

In No. 4 there was an excellent control of couchgrass.

Results from the Farm Scale Experiments were similar to those obtained in Small Plot Experiments in that no deleterious effect was observed on either soil type.

DISCUSSION

These results confirm the previous experimental work and it appears that kale will tolerate any small residues of amitrole-T which may remain after a spring application for couchgrass control. Furthermore experiment 4 confirms that the combination of kale and spring application of amitrole-T gives effective control of couchgrass.

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