Session 3A

The Management of Grass Weeds in Arable Crops

Chairman	Mr R WHITEHEAD
Session Organiser	Dr S R MOSS
Papers	3A-1 to 3A-6

INTEGRATED MANAGEMENT OF GRASS WEEDS IN ARABLE CROPS

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ABSTRACT

In most arable crops grass weeds have increased despite the use of effective grass herbicides. The main reasons for this increase are: crops favourable for grass weeds, reduced tillage, early sowing time, high nitrogen levels, and in some regions the development of herbicide resistant populations. Major grass weeds in Germany are *Alopecurus myosuroides, Apera spica-venti, Avena fatua, Echinochloa crus-galli, Elymus repens* and volunteer cereals. Since seeds of annual grasses have a short longevity they should not pose a major long-term problem if rotations and ploughing are introduced into the production system. Due to the slower population growth herbicide resistance should be delayed also. For the perennial grass *Elymus repens* in addition to chemical control, stubble cultivation and intercropping are quite effective. With the help of simulation models the effect of the various measures can be evaluated and control strategies developed.

INTRODUCTION

Grasses have always been part of the arable weed flora. However, in the last two decades populations of some species have increased considerably and pose serious problems in crop production. We know by experience, and it is also well recorded, that the composition of the weed flora is not constant. Weeds are strongly influenced by the production system, and changes in the weed flora can occur in a relatively short time (Mahn, 1984; Hilbig & Bachthaler, 1992). Many production systems today have two main characteristics: high intensity and restricted crop rotations. They are the rational response of the farmers to the present low price situation for agricultural products and the rather narrow spectrum of crops for which there is a market.

Most grass weeds are well adapted to present production systems: they respond extremely positively to reduced tillage, early sowing time, and high nitrogen levels (Pulcher-Häußling & Hurle, 1986; Knab & Hurle, 1988; Amann *et al.*, 1992). Their growth behaviour is also well adapted to many crops, and they are very competitive. Therefore they are controlled regularly. In most instances grass weeds are controlled with herbicides, and there are many effective products available. However, grass herbicides are expensive and in many crops they have to be used in addition to other herbicides, quite often as a separate application, or must be soil incorporated, both measures causing extra costs. Besides this economic aspect intensive herbicide use may lead to resistance in grass weeds (Niemann & Pestemer, 1984; Moss, 1987; Moss & Cussans, 1991) and this causes problems for both the users and producers of herbicides. Additionally there are also environmental problems especially with ground and surface water contamination creating great public concern and increasing political pressure on chemical weed control.

These problems are conducive to the development of an integrated management of grass weeds. Integrated management is a strategy which includes a combination of various indirect and direct control measures to keep the weed populations low. Integrated weed control strategies need basic information on the ecology and population dynamics of the species and the relationship between yield loss and weed density. In this paper some aspects of integrated control of grass weeds in arable crops will be reviewed with special regard to the situation in Germany.

OCCURRENCE, BIOLOGY AND IMPORTANCE OF MAJOR GRASS WEEDS

The major grass weeds to be considered are: *Alopecurus myosuroides*, *Apera spica-venti*, *Avena fatua*, *Echinochloa crus-galli*, *Elymus repens* and volunteer cereals. They are present in most parts of Germany. *Poa annua*, although occurring in all arable crops, sometimes at considerable densities, is not important as a weed due to its low competitiveness. In some regions with a high percentage of winter cereals and oil seed rape, *Bromus sterilis* is posing a problem, but at the moment it is restricted to a relatively small acreage (Kees & Pfeufer, 1984; Eggers, 1989). This is also true for *Cyperus esculentus* which occurs in some areas in maize and sugar beet (Gieske *et al.*, 1992) and is well known from the problems this adventitious plant has caused in the Netherlands (van Groenendael & Habekotté, 1988).

A. myosuroides germinates mainly in autumn, so it occurs mainly in early sown winter cereals and oil seed rape but it also occurs in spring cereals, sugar beet and other spring crops. The later the sowing time, the less *A. myosuroides* occurs. In maize it is not very common. *Apera spica-venti* germinates almost exclusively in autumn and therefore is a typical weed of winter crops, mainly winter cereals and very early sown spring cereals. In some areas there are mixed populations with *A. myosuroides*. *Avena fatua* germinates mainly in early spring and is very common in spring cereals and sugar beet, but it also occurs in winter wheat, especially if late sown. *Echinochloa crus-galli* germinates rather late in spring, so it is a typical grass weed in maize and, due to the expansion of this crop, is now present in most parts of Germany. There is a tendency for an increasing infestation in sugar beet. Volunteer cereals, mainly winter wheat in winter barley and winter barley in oil seed rape and to a far lesser extent in sugar beet, are quite common.

Although grass weeds show distinct differences with respect to time of germination, their seeds have a similar longevity in the soil: all are relatively short lived. Seed survival per year for *E. crus-galli* is about 30 % (Maun & Barrett, 1986), and for *A. myosuroides*, *A. spica-venti* and *A. fatua* it is 10 - 20 % (Rauber, 1978; Moss, 1985; Kaiser, 1989; Zwerger, 1993). For volunteer cereals the annual survival rate is estimated to be only 1 % (Rauber, 1988).

Seed production of grass weeds varies very much and depends mainly on the length of the vegetation period and number of tillers, which is strongly influenced by crop competition. On average *A. myosuroides* produces 100 - 200 seeds per plant in winter and spring cereals (Moss, 1990; Zwerger, 1993), *A. spica-venti* 1900 - 2000 in winter wheat (Warwick *et al.*, 1985; Kaiser, 1989), *E. crus-galli* 5000 - 7000 in maize (Maun & Barrett, 1986), and *A. fatua* produces only 10 and 30 seeds per plant in winter and spring cereals respectively (Rauber, 1978). For volunteer barley in sugar beet up to 40 seeds per plant are reported (Rauber, 1984).

The development of *E. repens* is strongly affected by the period of undisturbed growth and intensity of shading by the crop. Due to the longer growth period *E. repens* develops better in winter cereals than in spring cereals. However, the growth rate of this perennial plant is not very high in cereals, and rhizome bud formation occurs mainly after harvest if the stubble is not cultivated. In sugar beet and maize there is less competition and consequently conditions are more favourable for bud formation. The population growth rate in winter and spring cereals varies between 1.8 - 3.2 and 0.7 - 2.7 respectively (Hacker & Hess, 1986).

The damage caused by these grass weeds, expressed as the damage coefficient (crop loss [kg/ha] by 1 plant/m^2) is estimated to be 3 and 1.5 for *A. myosuroides* in winter and spring cereals, respectively (Pallutt, 1992) and 8 in oil seed rape (Küst & Heitefuß, 1990). For *A. spica-venti* it is 1 - 8 in winter cereals (Warwick *et al.*, 1985; Pallutt, 1992), and 4 and 8 for *A. fatua* in winter and spring cereals, respectively (Pallutt, 1992). For volunteer barley in oil seed rape the damage coefficient is estimated to be 15 (Munzel *et al.*, 1992). *E. crus-galli* can cause a 40 - 90 % yield reduction in maize (Brod, 1984), and *E. repens* up to 85 % in maize and 30 - 70 % in cereals (Werner & Rioux, 1977). Although these figures are approximations which depend on factors like crop density, sowing time, and nitrogen level, they give a rough idea about the crop loss these weeds can cause.

The importance of a weed can be derived from the acreage infested and the average degree of infestation, the crop loss, and cost of control. Table 1 gives an estimate of the importance of the six grass weeds in major arable crops in Germany. Cereals account for about 65 % of the total arable land, with 66 % of the cereal acreage being winter sown, 15 % is used for maize, 8 % for oil seed rape and 6 % for sugar beet. As Table 1 demonstrates, *A. myosuroides* has to be considered as the most important grass weed, followed by *A. spica-venti*, *E. repens*, and *A. fatua*. *E. crus-galli* and volunteer cereals have a significantly lower ranking.

TABLE 1. Importance of major grass weeds in various arable crops in Germany.

weeds	winter cereals	spring cereals	oil seed rape	sugar beet	maize
Alopecurus myosuroides	3	2	3	2	1
Apera spica-venti	3	1	1	0	0
Elymus repens	2	1	1	1	1
Avena fatua	1	3	0	3	2
Echinochloa crus-galli	0	1	0	2	3
Volunteer cereals	2	0	3	1	0

importance: 0 = no; 1 = low; 2 = medium; 3 = high

INTEGRATED MANAGEMENT

Three elements of a production system which show potential as indirect measures for the control of annual grass weeds are: crop rotation, tillage and sowing time. Growing crops which are unfavourable for a particular weed species will lead to a decline in infestation. Due to the short longevity of grass weeds in the seed bank this decline occurs relatively quickly. For example, *A. fatua*, which has been a major weed problem in Germany has become less important since the proportion of spring cereals has declined in favour of winter cereals. This in turn has increased *A. myosuroides* and *A. spica-venti* infestations.

Primary tillage has a very drastic effect on annual grass weeds. With ploughing the fresh seeds are buried for one season and small seeded species such as *A. myosuroides* and *A. spica-venti* have practically no chance of germinating and emerging until they are returned to the upper soil layer one year later. With reduced tillage they remain in the upper layer and contribute to the infestation immediately. Reduced tillage, which is very common now, certainly is a major reason for the present problems with grass weeds.

Delaying sowing time and taking advantage of the opportunity to control emerged plants by seedbed preparation should also reduce infestations, since later emergence of new seedlings is likely to be reduced.

The effect of these factors is demonstrated for *A. myosuroides*, as an example in Table 2. It shows clearly how winter wheat and oil seed rape favour this species. Reduced tillage results in at least a threefold increase compared to ploughing. Delayed drilling reduces infestation in the winter and spring crops, except for oil seed rape. In Fig. 1 the population dynamics of *A. myosuroides* in a maize-winter wheat-spring barley rotation is simulated. It illustrates the great importance of soil inversion in keeping the population low. It also indicates the effect of maize as a break crop. Crop rotations with a high proportion of spring crops and ploughing as the tillage system are not likely to run into serious problems with this grass weed.

TABLE 2. Effect of primary tillage and sowing time on *Alopecurus myosuroides* infestation (plants/m^2) in different crops. Weeds were not controlled (Amann, 1991).

	ploughing		reduced tillage	
	sowing time		sowing time	
crop	early	late	early	late
oil seed rape	46	27	125	131
winter wheat	51	9	307	58
spring barley	53	12	157	66
maize	9	1	33	6

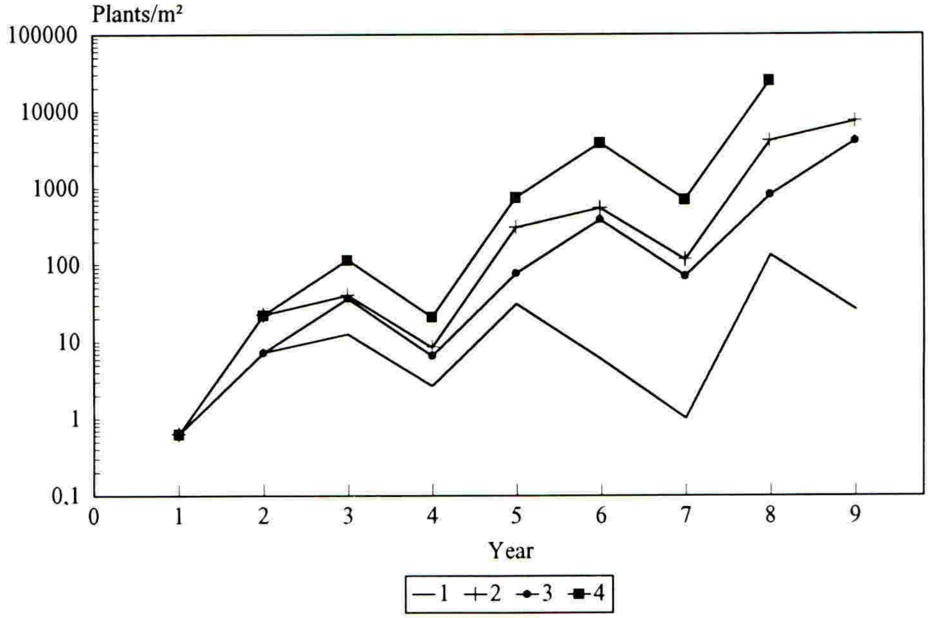

FIGURE 1. Effect of primary tillage on *Alopecurus myosuroides* infestation in a maize-winter wheat-spring barley rotation without weed control (Zwerger, 1993). 1 = ploughing for all crops; 2 = reduced tillage for winter wheat; 3 = reduced tillage for spring barley; 4 = reduced tillage for winter wheat and spring barley.

Regarding direct control measures in the crop, there is renewed interest in mechanical methods. In cereals harrowing, and in row crops inter-row hoeing, are established methods. They are not practised as a specific treatment for grass weeds, but for weeds in general. In cereals the control efficacy of *A. myosuroides* is about 50 % or less. In winter cereals, except later drilled winter wheat, harrowing has to be done early during the autumn, since weeds are most susceptible at early growth stages. However, the method is very dependent on weather and soil conditions, and sometimes there is hardly any control and additional herbicides have to be used. In general harrowing is expected to suffice if there are less than 50 *A. myosuroides* plants/m^2, which is usually the case in spring cereals. In sugar beet and other row crops inter-row hoeing is quite effective and only a band treatment with herbicides in the crop row is needed.

It must be emphasised that the control of *A. myosuroides* and other grass weeds is currently almost totally reliant on herbicides. For economic reasons, however, it is quite common to reduce the recommended dosage by 30 % or more without losing control efficacy significantly. A further contribution to a reduction in the use of herbicides is the concept of economic thresholds. In winter wheat the recommended threshold for *A. myosuroides* in Germany is 30 plants/m^2 (Niemann, 1981). But this concept is not fully accepted by the farmers. They probably believe it will result in heavy infestations in subsequent crops, which is of course, unjustified.

E. repens as a perennial responds differently to the annual grasses. With this species crop rotation is of less importance since it occurs in all main crops, and delaying drilling has practically no effect on the infestation. However, tillage is of great importance, and with reduced tillage this weed increases rapidly. It is well known that with regular stubble cultivation *E. repens* can be kept at a fairly low and tolerable level. Several passes with a cultivator or rotary cultivator gives reasonable control. Also growing a catch crop after cereal harvest is very effective if dense and suppressive stands are obtained. Table 3 shows that the catch crop (mustard) gave as good results as stubble cultivation in 1981. In 1979 however, the efficacy was very low because the mustard failed.

TABLE 3. Control of *Elymus repens* by stubble cultivation and catch crop growing; efficacy was measured in the following crop, maize. (Unpubl. data).

1979		1981	
treatment (no. of trials)	control efficacy (%)	treatment (no. of trials)	control efficacy (%)
stubble cultivation		stubble cultivation	
3 x (3)	73	3 x (2)	86
4 x (2)	76	4 x (1)	80
5 x (2)	87	5 x (1)	63
catch crop (7)	37	catch crop (4)	82

CONCLUSIONS

The major annual grass weeds offer good opportunities for the development of integrated management systems because they are rather specialized in certain crops and their seeds have a short longevity in the soil seed bank. Simple changes in cultural methods can have significant effects on grass weed populations, but these methods are rarely used by farmers. At present they still control grass weeds quite successfully with herbicides. The reasons for this are the availability of grass herbicides for all major crops, the good efficacy, and the high reliability. Cultural methods usually are less effective and reliable, but contribute to a lower infestation and consequently less herbicide use. Chemical control will continue to play a dominant role in modern agriculture, especially for control of grass weeds. An integration of cultural and chemical methods however, is likely to delay the build up of herbicide resistance and reduces environmental problems with these chemicals. What combination of methods is appropriate for a given situation can be determined best by simulating the population dynamics of the weed species in question and investigating how it is affected by various direct and indirect control methods. To run such simulations sufficient data must be available to describe the field situation in a realistic way. It is a challenge for weed research to elaborate and improve such data for the major grass weeds. This approach will certainly help to increase the acceptance of an integrated strategy by farmers.

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THE *POA* SPECIES: PROBLEMS AND MANAGEMENT IN DANISH ARABLE FIELDS

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ABSTRACT

Poa spp. are common and can cause serious problems in Danish crops. During the last two decades, *P. annua* has shown decreased frequency in winter rye and clover/grass leys, but rather constant frequency in oilseed rape, spring barley and winter wheat. It is still the second most common Danish weed. Prohibition of straw-burning favours straw incorporation that may increase soil water capacity and in turn the frequency of *Poa* spp. The increasing area of winter cereals also favours annual grasses. Several grass herbicides can control *Poa* species, but legislation to reduce herbicide use will require their use to be integrated with cultural control measures.

INTRODUCTION

Three *Poa* species are common on Danish arable land. Two of these species, *Poa pratensis* (smooth meadow-grass) and *Poa trivialis* (rough meadow-grass), are economically important crops for grass seed production, but may act as weeds in other arable crops. The third species, *Poa annua* (annual meadow-grass), is an annual weed in spring or autumn sown crops; it is the second most common weed in crops, only *Stellaria media* (chickweed) is more frequent.

Although the presence of *P. annua* rarely causes a significant economic loss in arable crops, it can cause problems in grass seed crops because the seeds are difficult to separate. It can be a nuisance also in fields with lodged crops because it makes harvest difficult.

During this century four extensive weed surveys have been carried out. The two most recent ones were in 1966–1970 and in 1987–89 and can be used to evaluate changes in the frequency of the *Poa* species. In addition, the survey data can be used to investigate the relationships between the frequency of the weeds and soil properties and plant nutrients. These relationships may enable us to predict changes in weed frequencies due to changes in land use.

This paper will review the changes in frequencies of *Poa* species, especially *P. annua* between the last two surveys, and show that the changes in agronomic practices have caused changes in the frequency of these species. Agronomic practices continue to change, some are required by legislation, and we make predictions of the likely consequences of these on *Poa* frequencies. Finally, we discuss the interaction of chemical and non-chemical methods that will be needed to manage these weeds in the future. In addition, control methods in Danish arable crops will be discussed.

FREQUENCIES IN ARABLE CROPS

In 1966–1970 the frequencies of weed species in 466 fields growing 19 different crops were surveyed. The analysed fields were in conventional agricultural use, and plots on the fields were intentionally left totally unsprayed in the survey year. Sampling was carried out in July and August. The frequencies of weed species were recorded by listing the presence or absence of the species in ten randomly selected circular sample plots of 0.1 m^2 within each field (Raunkiær, 1934). In addition, the presence or absence of the species on the fields were recorded. The fields were located throughout the country and can be assumed to reflect the weed situation in Denmark twenty years ago (Haas & Streibig, 1982). A similar survey, which was conducted on unsprayed plots in 1987–1989, included 357 fields growing 9 different crops (Andreasen *et al.*, 1991a, 1993).

The frequency of *P. annua* was relatively high in all crops (Table 1), but in only two of the five crops common to both surveys was there a significant reduction in frequency. Note that the frequency was much lower in sugar beets than in fodder beets. In the survey of 1987–89 the frequency of *Poa trivialis* varied from 0 to 1.3% based on 0.1 m^2 samples, and it was found in 10.7% of all fields. The frequency of *Poa pratensis* was 0.2% in spring barley and sugar beet and it was found in 3.1% of the fields.

TABLE 1. Frequency of *Poa annua* in Danish crops. Data from Andreasen *et al.* (1991a, 1993).

Crop	Frequency (%) 1966–70	% of total arable area 1966–70	Frequency (%) 1987–89	% of total arable area 1987–89	Change in frequency[1]
Fodder beet	no data	4.0	50.6	4.0	
Sugar beet	no data	2.9	9.8	2.4	
Peas	no data	0.2	43.6	5.8	
Spring oilseed rape	48.3	0.3	40.2	7.1	n.s.
Spring barley	33.7	41.7	32.3	35.8	n.s.
Winter barley	no data	0.0	31.3	2.0	
Winter wheat	42.9	2.4	38.1	13.4	n.s.
Rye	42.4	1.3	22.1	4.0	--
Clover/grass leys	67.3	17.2	46.8	9.2	-

[1]) n.s. = no significant change; - = significant decrease, (P<0.05); -- = significant decrease, (P<0.01).

CONTAMINATION OF GRASS SEED

Poa annua contaminated 86% of all *P. trivialis* seed lots examined between 1966 and 1969, with an average of 2900 seeds per kg (Olesen & Jensen, 1969). Since then crop management and seed cleaning methods have improved and certified seed needs to meet the EEC standards of a maximum 2% by weight of other species in seed lots and one species is allowed to account for a maximum of 1%. As a consequence of improved methods and standards, the 153 samples of *P. trivialis* seed, tested between 1980 and 1983, had less than 1.8% contamination with *P. annua*. No decisive changes in the content of *P. annua* have appeared since 1980–1983. Contamination of *P. pratensis* by *P. annua* was similar to that in *P. trivialis* (Jensen, 1989). Sometimes *P. pratensis* contaminates *P. trivialis*, but it was found in less than 0.2% of the samples (Jensen, 1984).

RESPONSE TO NUTRIENTS AND SOIL FACTORS

The frequency of *P. annua* was influenced by crop type and increased with increasing soil organic matter, with a significant interaction (Andreasen *et al.*, 1991b). It is likely that the organic matter increased the weed frequency through improvements in the water holding capacity of the soils. Soil factors such as pH, available phosphorous, exchangeable potassium, magnesium and manganese did not seem to have any effect on the occurrence of *P. annua*. This indirect effect of organic matter may increase the frequencies of *P. trivialis* and *P. pratensis* also, since they too are favoured by humid growing conditions (Andreasen *et al.*, 1991a, 1991b).

CONTROL OPTIONS: CHEMICAL

Phenoxy acids and sulfonylureas have been used extensively for weed control in cereals in Denmark, but these herbicides do not control *P. annua*. However, several graminicides are registered in Denmark which control *Poa* species (Table 2).

TABLE 2. Grass herbicides registered for control of *Poa* species in Denmark. Control at recommended rates: +++: Good, ++: Fair, +: Weak (Anonymous, 1993a, 1993b, 1993c).

Herbicide	*Poa annua*	*Poa trivialis*	*Poa pratensis*
Isoproturon	+++	+++	++
Pendimethalin	+++	++	++
Methabenzthiazuron	+++	+++	++
Haloxyfop	+++	+++	+++
Propyzamide	+++	+++	+++
Fluazifop	+	+++	+++
Glyphosate	++	+++	+++

Herbicides that contain isoproturon provide good control of *Poa annua* in spring and winter wheat, barley and rye. Pendimethalin can be used in winter cereals and peas, but should not be used on sandy soils or soils rich in organic matter because of the risk of crop damage.

Methabenzthiazuron is registered for winter cereals, spring wheat and grass seed crops. Haloxyfop has recently been registered in Denmark and controls all three *Poa* species in most broadleaved crops including oilseed rape and beets, and is selective in red fescue (Mondrup, 1993). Trifluralin controls *P. annua* in oilseed rape and peas. Propyzamide can be used in grass, clover, oilseed rape and seed crops. Fluazifop, which can be used in broadleaved crops and red fescue, gives good control of *P. trivialis* and *P. pratensis*, but is not suitable for *P. annua* control.

Herbicides with broader weed range can be used for control of *P. annua* in crops such as beets (metamitron) and potatoes (metribuzin). Glyphosate can be used as a desiccant in cereals, oilseed rape, peas and grass, and may at the same time be used to control *Poa* species. This may be especially beneficial, where *Poa* infestations make combine-harvest difficult.

In summary, several herbicides give good control of *Poa* species in almost all major Danish crops, but herbicide costs may sometimes exceed yield benefits.

CONTROL OPTIONS: NON-CHEMICAL

A number of control methods are known to control grass weeds. The fibrous root system of seedlings are easily dislodged by cultivation and harrowing. Any cultural, or chemical, method that prevents seed production has benefits since the seed reserves in the soil do not persist for long periods (Roberts & Dawkins, 1967)

After harvest harrowing, without ploughing, is an effective method to control grass species which generate from seeds that escaped harvest. This method prevents seeds from being deeply buried in the soil and from causing problems in the future (Thorup, 1988). Seed populations of *P. annua* in soil decreased exponentially under both disturbed and undisturbed conditions (Roberts & Dawkins, 1967). The annual rate of loss in undisturbed plots was 22% compared with 30% and 36% where two and four cultivations were given.

Crop type and rotation are generally considered important methods of controlling weeds. While the frequency of *P. annua* varies among crops (Table 1), little is known about the response of *Poa* species to crop rotations. As *P. annua* is shade tolerant, it may survive the competition from many crops and maintain production of viable seed, although at low numbers.

DISCUSSION

Although earlier investigations have stated that *P. annua* is increasing in frequency (Bakkendrup-Hansen, 1969), the two last Danish weed surveys do not support the view that this weed has increased during the last two decades. Our hypothesis about a decreased frequency could only be supported for rye and clover/grass leys. Unfortunately, it is not possible to investigate frequency changes in peas, winter barley and beets, because these crops were not represented in the survey 1966–1970. A general decrease in some crops may mask increases in some fields, and decrease in others. The reported increased incidence, at least in some areas, may be caused by term long use of phenoxy acid herbicides rather than newer and more effective grass killers.

P. annua ranks as the second most frequent weed in Danish crops, and in some it may be increasing. The only crop with low incidence of *P. annua* was sugar beet. This exception may be due to the tradition of intensive weed management, combining mechanical and chemical methods, in sugar beet growing areas, resulting in small seed pools. The low frequencies of *P. pratensis* and *P. trivialis* in the 9 crops surveyed make them an insignificant problem in Danish crops, although the species can be troublesome and economically important as impurities in some samples of grass seed.

At present herbicides, perhaps in combination with cultivation when possible in the rotation, seem to control *Poa* species. It should be noted, however, that herbicide resistance may be a concern, especially if herbicides with the same mode of action are used repeatedly. In Denmark like other countries, triazine-resistant populations of *P. annua* have been found (Jensen, 1993), but no resistance to other grass herbicides has yet been discovered in Denmark.

Because of the Danish 'green crops' legislation aimed to reduce nitrate leaching, crop rotations are currently changing towards increased winter cropping, including winter cereals and oilseed rape. Such changes are assumed to favour annual grasses such as *Poa* species, and at the same time, mechanical control is becoming more difficult due to the short time between harvest and sowing. The prohibition of straw-burning on Danish fields since 1991 has encouraged farmers to use straw incorporation, which may increase the water holding capacity and organic matter of soils; both increases can favour *P. annua* (Andreasen *et al.*, 1991b).

Recent EEC set-aside initiatives may also affect annual grass species, especially when the fields are out of cultivation for only one year. These fields appear to be 'weed seed factories' for annual weed species. On the other hand, set-aside for several years encourages perennial species at the expense of annual weeds. At present, it is unclear which practice will be required by the EEC in the future.

More intensive chemical control is not the answer to this expected increase of grass weeds. The Danish action plan requires a 50% reduction in pesticide use by 1997 compared to 1981–85. Both the quantity of sold active ingredient and the so-called spraying intensity must be reduced (Haas, 1989). The spraying intensity is defined as the total number of times a crop is sprayed with recommended pesticide rates within a growing season. Another reason is the lower prices for agricultural products in the EEC, which tend to discourage the use of expensive chemical control measures.

The legislation requiring re-evaluation of all old herbicides in Denmark has resulted in withdrawal, or banning, of several products. These changes hit the Danish grass seed producing industry hard (Olsen, 1993). The increasing costs to get new products registered in Denmark makes the agrochemical companies give a low priority to the development and registration of new grass herbicides for use in grass seed fields.

To meet these government-directed initiatives, control decisions must be based on an evaluation of the expected costs and benefits. Where herbicides are used, dosages should be adjusted to the weed flora present, the developmental stage of weeds, and environmental conditions. Also, cultivation and cultural practices should be integrated with the chemical methods.

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DETERMINATION OF ECONOMIC THRESHOLD POPULATIONS OF POA ANNUA IN WINTER CEREALS

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ABSTRACT

Five seed rates of *P. annua* were broadcast over recently drilled winter wheat crops on four sites in England and Wales in 1989, 1990 and 1991. The resulting *P. annua* populations were controlled either (a) pre-emergence, (b) at the 1-3 leaf stage or (c) the 2-4 tiller stage. Pre-emergence treatment produced the best control of *P. annua*, 98%, in April and the best overall yield response of 0.78 t/ha (10%). Up to half of this yeild response was derived from broadleaved weed components. The calculated overall economic threshold for *P. annua* under the experiment parameters was 714 plants/m². Under higher *P. annua* populations and the wetter climate of South Wales the calculated economic threshold was lower at 416 plants/m².

INTRODUCTION

Poa annua (annual meadow grass) flower initiation is independent of day length and vernalisation so it can flower at any time of the year. Normally however it germinates in late summer from seed produced one or two months earlier. Young seedlings grow rapidly in the autumn and begin active growth earlier in the spring than most other grasses (Wells & Haggar 1974). Germination percentage increased rapidly as soil moisture content increased from 20 to 40 per cent (Koch, 1968).

Seed populations in the soil have been recorded at 90 million per hectare following grass clover leys (Roberts, 1963) and five million per hectare after five years of horticultural cropping with effective weed control. (Roberts & Stokes, 1966). Only *Stellaria media* (chickweed) exceeded this frequency in that study.

Loss of seeds in the soil has been measured at 22% per year (Roberts & Dawkins, 1967) and 30-36% per year where two to four cultivations take place. However the potential for multiplication and replenishment of the soil seed bank after uncropped set-aside is likely to be dramatic.

In ADAS trials between 1979 and 1983 good control of poa species was achieved but did not result in direct yield responses even at panicle populations of 3390 m² at harvest. (Harvey 1985). He identified the increased importance of *P. annua* in the higher rainfall areas.

In later ADAS trials pre-emergence treatments gave most consistent control of *Poa* species, averaging 96% control with an average yield response of eight per cent over controls over 3 years (Sherrott, 1988). Additional benefits of easing harvest and reducing the "green bridge" carry over of diseases and pests were also noted.

Survey data (Davis et al, 1990) indicated that in excess of 1,600,000 hectares of cereals were treated with broad spectrum graminicides for control of grass weeds. The introduction of strict limits on herbicide residues in ground water, and an increase in non competitive set-aside regimes will increase the importance of decision making thresholds for control of *Poa* species.

The object of this experiment was to test the feasibility of establishing economic thresholds for control of *P. annua* in differing climatic locations and to determine the effect of removal of *P. annua* on the nitrogen uptake and yield of winter cereals.

MATERIALS AND METHODS

Four experiments were carried out each harvest year in 1990, 1991 and 1992. Experiments were located in Northumberland, Humberside, Norfolk and South Wales. Plot sizes of 20 m x 1.25 m were used on the South Wales sites and 12 m x 2 m on all other sites. All treatments were fully randomised with three replicates.

P. annua seeds were broadcast at 0, 2000, 4000, 6000 and 8000 seeds/m² onto farm drilled wheat crops using Ojyord plot drills with tubes removed from the coulters. Broadcasting took place with 1 or 2 days of drilling wheat.

Herbicide treatments a-d were applied to all seeding treatments:-

(a) Pre-emergence - Diflufenican (DFF) + isoproturon (IPU) 'Panther' at 0.1 + 1.0 kg AI/ha.

(b) Early post emergence - *P. annua* at 1-3 leaf stage - DFF + IPU as (a).

(c) Later post emergence - *P. annua* at 2-4 tillers - IPU 2.07 kg AI/ha.

(d) Untreated controls.

Additional herbicide treatments were applied to 4000 *Poa* seeds/m² plots in 1991 and 1992 only:

(e) DFF + trifluralin 'Ardent' 0.1 + 1.0 kg AI/ha.

(f) Pendimethalin + IPU 'Encore' 1.0 + 0.5 kg AI/ha.

(g) Trifluralin + isoxaben 'Tristar' + 'Flexidor' 0.95 + 0.077 kg AI/ha.

(h) Pendimethalin + simazine (AC 3010) 0.75 + 0.25 kg AI/ha.

(i) DFF + IPU 0.094 + 1.5 kg AI/ha as 'Javelin' + IPU.

P. annua populations were assessed as plants/m² 6 weeks after sowing and in late March-early April and as per cent ground cover at harvest. Assessments were made using 0.25 x 0.25 m quadrats with 5 or 10 counts per plot depending on evenness of weed establishment. Samples of wheat and *P annua* were collected from the 8000 *P. annua* seeds/m² plots of treatments (b) and (d) at GS 30-31 (Tottman, 1987) in March-April and at GS 60-61 in June 1991 and 1992 only and analysed for dry matter and nitrogen uptake by wheat and *P. annua*.

Broad leaved weeds were controlled using contact herbicides in spring or residual broad leaved weed herbicide isoxaben, at drilling. With the exception of South Wales 1990 all trials were harvested by combine harvester.

RESULTS

Establishment of *P. annua* populations ranged from 0 to 3370 plants/m² (Table 1) with lower levels on the drier Norfolk site and higher levels on the South Wales site. The Northumberland and Humberside sites also produced high *P. annua* populations one year in three.

Broad leaved weeds also established significant populations; on the South Wales site in 1990 - *Aphanes arvensis* and *Thlaspi arvense*; the Northumberland site in 1991 and 1992 *Stellaria media*. Humberside in 1992 *Stellaria media* and *Matricaria perforata;* Norfolk site in 1992, *Bilderdykia convolvulus, Capsella bursa-pastoris* and *M. perforata.*

P.annua Control

Pre-emergence treatment (a) gave consistently the highest levels of control, around 97 to 98% over all *Poa* seed rates in April (Table 2). This treatment maintained control through to harvest giving a mean of 91% reduction in ground cover over all *Poa* seed rates. Early post-emergence treatment (b) gave 78 to 87% control in April and a mean of 70% control over all sites and seed rates at harvest. Later post-emergence IPU, treatment (c), gave more variable results in April assessments 53-62% control. On some sites death of *P. annua* was not complete on the April assessment date. Harvest assessments showed rather better control levels for this treatment of 75% over all sites and *Poa* seed rates. Treatments (e) to (i) gave mean *P. annua* control at harvest of 73, 82, 89, 95 and 89% respectively.

Yield Responses

At the levels of *P. annua* control achieved (Table 2) there were no detectable yield penalties from established *P. annua* populations within each herbicide treatment. The overall mean yield response to herbicide treatment across all *P. annua* populations was (a) 0.78 t/ha (10%); (b) 0.62 t/ha (8%) and (c) 0.45 t/ha (6%). Yield responses of treatments applied to the 4000 *P. annua* seed rates were (a) 0.75, (b) 0.66, (c) 0.65, (e) 0.73, (f) 0.98, (g) 0.66, (h) 0.82, (i) 0.98 t/ha.

Significant yield responses were obtained on a number of sites in the absence of established *P. annua* populations (Table 3). Indigenous *P. annua* populations were relatively low on most sites and it is likely that the yield responses achieved on unseeded plots were in part due to control of broadleaved weeds.

In the absence of herbicide treatment mean yield reduction from the 8000 *Poa* seed/m² plots was 0.52 t/ha (Table 4) from an increase in *P. annua* population of 1476/m², a yield reduction of 0.035 t/ha per 100 *P. annua* seeds/m². At typical herbicide costs of £24 per hectare and feed wheat prices of £95/t a yield increase of 0.25 t/ha is required to recoup herbicide costs. From the above mean yield response a population of 714 *P. annua* plants per m² would be required to recoup typical herbicide cost.

Measurements of wheat and *P. annua* biomass and nitrogen content at GS 30-31 and 60-60. showed that *P. annua* could account for up to 17% of total nitrogen uptake at this time.

It was not possible in these experiments to measures the effect of high *P.annua* populations on slowing down harvesting operations or on increasing grain moisture content at harvest.

TABLE 1. Number of *P. annua* plants established per square metre in April.

Seed Sown/m²	Northumberland 1990	1991	1992	Humberside 1990	1991	1992
0	5	139	34	19	7	0
2000	200	299	1400	271	823	200
4000	219	704	2462	550	1565	353
6000	310	887	2863	706	2179	422
8000	498	1076	3290	854	3334	537

	Norfolk 1990	1991	1992	South Wales 1990	1991	1992
0	5	40	83	37	232	86
2000	63	291	168	352	909	1125
4000	98	472	352	603	1002	1776
6000	240	915	402	811	1461	1854
8000	283	704	902	880	2667	3371

TABLE 2. Per cent Control of *P. annua* April

Treatment	Seed Sown/m²	1990	1991	1992	Mean
a	0	95	99	100	98
	2000	98	99	96	98
	4000	98	99	97	98
	6000	98	99	94	97
	8000	98	98	96	97
b	0	64	95	98	86
	2000	75	85	92	84
	4000	75	90	95	87
	6000	60	93	91	81
	8000	51	89	94	78
c	0	48	62	50	53
	2000	57	79	51	62
	4000	46	77	61	61
	6000	49	73	40	54
	8000	33	83	47	54

TABLE 3. Yield response to herbicides on unseeded *P. annua* treatments t/ha (85% DM) compared with untreated yield (d). SED calculated on actual treatment yields.

Herbicide Treatment	Northumberland 1990	Northumberland 1991	Northumberland 1992	Humberside 1990	Humberside 1991	Humberside 1992	Norfolk 1990	Norfolk 1991	Norfolk 1992	South Wales 1990	South Wales 1991	South Wales 1992	Mean
a	1.85	0.30	0.75	1.12	-0.06	-1.36	0.93	0.23	0.92	NA	0.09	-0.23	0.41
b	0.88	0.61	0.54	1.10	0.61	-0.61	0.49	0.21	0.71		0.07	-0.74	0.35
c	0.19	0.90	-0.18	1.25	0.99	-0.95	0.10	-0.66	0.48		-0.25	-0.35	0.14
d	8.90	7.87	7.51	7.71	9.49	8.89	5.91	8.57	6.20		11.72	7.18	8.18
SED±	0.45	0.29NS	0.31	0.49	0.45NS	0.77NS	0.25NS	0.29NS	0.26		0.34	0.31	

TABLE 4. Yield depression from increasing *P. annua* populations compared with unseeded t/ha (85%) SED calculated on actual treatment yields.

Poa seed/ m²	Northumberland 1990	Northumberland 1991	Northumberland 1992	Humberside 1990	Humberside 1991	Humberside 1992	Norfolk 1990	Norfolk 1991	Norfolk 1992	South Wales 1990	South Wales 1991	South Wales 1992	Mean	Poa Plants/ m² April
0	8.90	7.87	7.51	7.71	9.49	8.89	5.91	8.57	6.20	NA	11.72	7.18	8.18	57
2000	-0.07	-0.27	+0.52	+0.66	-0.30	-1.09	+0.15	-0.42	+0.55		-1.11	-0.24	-0.15	508
4000	-0.11	-0.21	-0.31	-0.33	-0.59	-1.01	-0.24	+0.14	-0.12		-2.12	-0.57	-0.50	846
6000	-0.18	-0.44	+0.36	-0.13	-0.07	+0.02	+0.14	-0.42	+0.52		-2.07	-0.55	-0.26	1088
8000	-0.54	-0.27	+0.43	-0.48	-1.09	-0.74	+0.17	-0.01	+0.06		-2.77	-0.73	-0.54	1533
SED±	0.45	0.29NS	0.31	0.49	0.45NS	0.77NS	0.25NS	0.29NS	0.26		0.34	0.31		

DISCUSSION

The level of *P.annua* control recorded in April, Table 2, was similar for each *P.annua* population established. This inevitably means higher *Poa* survival numbers from higher initial *P.annua* populations.

In spite of specific treatments to selectively control broadleaved weeds it proved impossible on most sites to eliminate the effect of these weeds on wheat yields. The results have been presented therefore to show the response to herbicide treatments in the absence of sown *P.annua* populations Table 3 and the yield depression from increasing *P.annua* populations in the absence of herbicide treatment. The responses are not directly additive but reflect the normal farm situation that weed thresholds cannot be considered in isolation. It was clear from observations that development of *P.annua* can be suppressed by a vigorous wheat canopy and also by competition from broadleaved weeds such as chickweed.

Dry matter production and nitrogen content of wheat and *P.annua* during the growing season indicated that *P.annua* could account for up to 17% of total nitrogen uptake. In Table 4 the overall yield depression was in the order of 6% from high populations of *P.annua* but reached 24% on one site in South Wales. It is clear from the results that the economic threshold will vary with site, year, product and dose rate. Where *P.annua* growth was most competitive on the South wales sites the calculated economic threshold was reduced from the overall mean of 714 *P.annua* plants/m^2 to 416 plants/m^2. Conversely on the drier Norfolk site *P.annua* populations established failed to produce an economic response.

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EFFECT OF TEMPERATURE AND HOURS OF SUNLIGHT ON THE EMERGENCE OF *BROMUS* SPP. AND IMPLICATIONS FOR WEED CONTROL

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ABSTRACT

Bromus spp. (Bromes) are increasingly significant weed problem in Spain. This study evaluates the effect of temperature and sunlight hours on the seedling emergence of three *Bromus* species from twelve world agroclimatic origins, under non-limiting moisture conditions. A factorial variance analysis evaluated the relations between the planting dates and origins with the characteristics of emergence. The thermal integrals to reach emergence and the emergence time for a particular time of year, varied among species, suggesting that these factors could be genetically controlled. Nevertheless, since the temperatures had a large influence on emergence, a genotype-environment interaction was suggested. The results suggest that *Bromus* spp. are adapted to the climate of Spain. To achieve a rapid and effective reduction of the weed on farms, a cropping system that facilitates uniform and rapid germination of these weeds should be implemented.

INTRODUCTION

Bromus spp. (Bromes) are annual grasses of Mediterranean origin, which are widespread in winter rainfall pastures and are important weeds of winter cereals in different world agroclimatic zones. Since the beginning of the 1980s, *B. diandrus* L. (the great brome) has become an increasingly significant problem in Spain (Garcia-Baudin, 1984; Riba *et al.*, 1990).

The rapid spread of *Bromus spp.* in recent years, due to the early sowing of rainfed crops and to the lack of effective herbicides for selective control has produced, in some cases, substantial crop losses. As a consequence, this problem may be a threat to the development of agronomic systems which aim to minimize management inputs.

The presence of *Bromus spp.* as weeds in different agroclimatic zones may be due to the great genetic variability of the species or to phenotypic plasticity. A knowledge of the physiological variations in *Bromus spp.* of different origins is essential to understand their adaptation to different environmental conditions and to be able to develop control strategies.

This study evaluates the effect of temperature and time of sowing on the emergence of seeds of *B. diandrus* (Great brome), *B. rigidus* (Ripgut brome) and *B. sterilis* (Barren brome) from twelve different origins. The main objective was to determine whether the characteristics of emergence of *Bromus* from different origins could be responsible for their great adaptation to a varied range of environmental conditions.

MATERIALS AND METHODS

Vegetal Material

Seeds of three *Bromus* species were collected from twelve agroclimatic world areas: *B. diandrus*, from the Iberian peninsula (Cordoba (CD), Palau d'Anglesola (PA) and Coruche (CR)), Italy (Corse (CO)), France (FR), Morocco (MO), Australia (AU) and USA (Oregon (OR)); *B. rigidus*, from Morocco (MO) and Australia (AU); and *B. sterilis* from Italy (Corse (CO) and France (FR). All origins are characterized by a Mediterranean-type climate with strong seasonality of rainfall and temperature. In order to homogenize and multiply samples, all seeds were sown in a glasshouse the year previous to the experiment, collected the following May and maintained at temperature of 25 to 30 °C.

Experimental design and evaluation of emergence

All experiments were conducted at the CIT-INIA experimental station (40° 29' N; 3° 29' W). To eliminate the influence of rainfall, the experiment was carried out in an open-side glasshouse, without additional lighting, in which the outside temperatures were not modified. The experimental design was based on samples of fifty seeds of each species and origin, each planted (0.5 to 1 cm deep) in an individual jiffy-pot (3x3 cm). Water was provided (from above) so germination could occur under non-limiting moisture conditions. Eight different planting dates (31 Oct., 15 Nov., 2 Dec., 16 Dec., 3 Feb., 17 Feb., 3 Mar., and 17 Mar.) representing different environmental conditions (from October to March) were evaluated.

TABLE 1. Average maximum (Tmax) and minimum (Tmin) temperatures ° C and number of days with temperatures below 0° C during the period of time of the experiments in the experimental station of CIT-INIA.

	Tmax °C	Tmin °C	Days with $t \leq 0°$ C	SR $MJ.m^{-2}.day^{-1}$
November	14.5	0.6	15	6.98
December	11.2	-1.8	21	6.99
January	9.3	-5.9	30	7.85
February	13.5	-4.6	24	8.78
March	17.9	-0.8	20	16.45
April	20.9	2.7	0	19.54

Daily maximum and minimum temperature and hours of sunlight (measured solar radiation, SR in $MJ.m^{-2}.day^{-1}$) were recorded (averages are shown in Table 1). Daily observations of seed emergence were taken. Each experiment ended in May. For each sample and each sowing period, the following parameters were evaluated: total number of plants that emerged; emergence time (≤ days to emergence of each seedling/number of seedlings, ET); days to the emergence of the first seedling; days to the emergence of 50% of the seedlings (E50); emergence interval (days from the emergence of the first seedling to the last, EI); and thermal integrals with base 0, 3 and 5 °C (≤ (daily average temperatures-base) during the period from sowing to emergence). A factorial variance analysis evaluated the effects of different planting dates and origins in the emergence characteristics.

RESULTS AND DISCUSSION

The emergence of *Bromus* of different origins was evaluated on different dates of sowing that gave different conditions of temperatures and daily sunlight hours for germination. The total percent of emergence was high in all *Bromus* at all dates, (above 90%), except in mid December. As it is generally assumed that there is a lack of seed dormancy in all *Bromus* species (Gill and Castairs, 1988; Serrano *et al.*, 1992), the factors that inhibit germination are usually considered to be high temperatures and hydric stress. It is known that the seeds of *Bromus* are not as photosensitive as other seeds of other grass weeds, although light may inhibit germination to some extent (Jauzein, 1989).

TABLE 2. Days to the emergence of 50% of the seedlings (E50) for the different species and origins of *Bromus* on the different sowing dates.

Species and Origin	Sowing Date							
	31 Oct	15 Nov	2 Dec	16 Dec	3 Feb	17 Feb	3 Mar	17 Mar
B. diandrus								
Cordoba	13	21	18	56	28	20	13	8
Coruche	14	20	19	59	28	20	13	8
Palau	14	24	21	63	28	20	14	9
Australia	13	21	21	57	28	20	15	10
Corse	13	22	18	56	28	20	13	8
France	14	22	24	64	28	20	14	10
Morocco	14	21	18	64	28	20	13	9
Oregon	13	21	18	70	28	20	14	9
B. rigidus								
Australia	18	31	36	70	32	20	17	16
Morocco	15	25	31	77	29	20	15	13
B. sterilis								
Corse	12	24	19	79	28	20	13	10
France	14	26	20	88	28	20	14	13

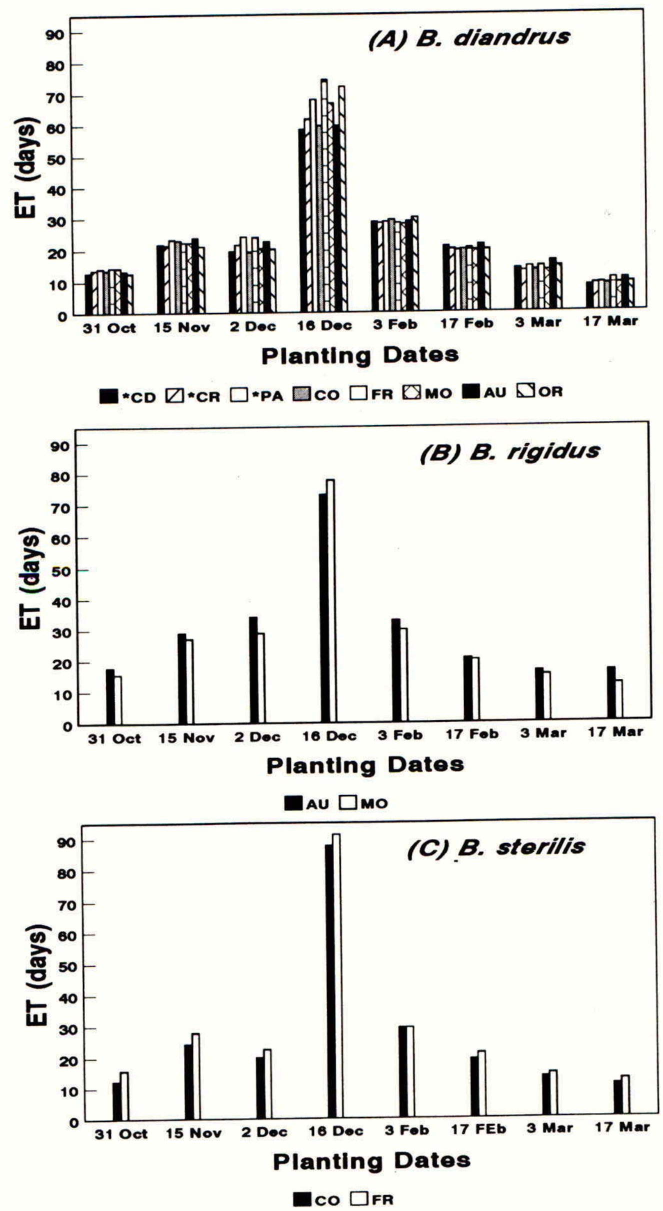
TABLE 3. Average values of the thermal integrals with base 5°C from sowing to emergence for the different species and origins of *Bromus* on the different sowing dates.

Species and Origin	Sowing Date							
	31 Oct	15 Nov	2 Dec	16 Dec	3 Feb	17 Feb	3 Mar	17 Mar
B. diandrus								
Cordoba	46	51	20	8	26	46	57	45
Coruche	51	51	20	10	20	40	51	43
Palau	51	51	20	16	26	40	57	43
Australia	46	53	20	8	26	46	68	43
Corse	46	53	20	8	26	40	51	43
France	46	51	20	19	20	40	57	43
Morocco	46	51	20	16	20	40	51	43
Oregon	42	46	20	18	26	40	57	43
B. rigidus								
Australia	76	53	20	19	36	40	68	45
Morocco	68	53	20	31	30	40	62	43
B. sterilis								
Corse	42	53	20	65	36	35	51	43
France	51	52	20	76	26	46	57	43

TABLE 4. Significance level ($p \leq 0.05$) of the variance analysis for the different planting dates and origins of *Bromus* spp. Significant (S); Non-significant (NS). Emergence time (ET); emergence interval (EI); 50% of seedling emergence (E50); thermal integrals with base 0, 3 and 5 ° C (I0, I3 and I5, respectively).

	ET	EI	E50	I0	I3	I5
Planting Date	.0001 S	.0001 S	.0001 S	.0001 S	.0001 S	.0001 S
Origin	.0037 S	.5468 NS	.0005 S	.0209 NS	.0429 NS	.2002 NS

FIGURE 1. Average Emergence Time (ET) for the different species and origins of *Bromus* on the different sowing dates. (A) *B. diandrus*; (B) *B. rigidus*; (C) *B. sterilis*. Cordoba (CD), Coruche (CR), Palau d'Anglesola (PA), Cose (CO), Francia (FR), Morocco (MO), Australia (AU), Oregon (OR). * Origins of the Iberian peninsula.



In general, there was a great homogeneity in the emergence parameters for all samples for each particular sowing date (Figure 1 and Tables 2 and 3). The variance analysis (Table 4) shows that the sowing time was significant (5% level) for all variable analyzed. Nevertheless the origin was only significant for the emergence time and the emergence of 50% of the seedlings.

The average emergence time for the three species of *Bromus* for the different sowing times, (Figure 1) shows the great influence of the sowing time and the great homogeneity among species and samples from different geographical origins, except in the sowing of mid December, in wich the averaged emergence time ranged from 58 to 91 days (Figure 1); this was the period with more days with temperatures below 0° C and low sunlight hours (Table 1). In these non-favourable temperature conditions there is a different plant response, seedling emergence in *B. sterilis* is slower than in the case of *B. diandrus* and *B. rigidus*.

In the sowing dates with low levels of solar radiation (31 October, 15 November, and 2 December), the emergence is comparable to that in other sowing periods. Therefore, our data suggest the low sensitivity of *Bromus* seedling emergence to light, in agreement with Jauzein (1989).

The time to reach emergence for a particular time of year, varied between species, suggesting that this characteristic could be genetically controlled. Nevertheless, since sowing times had a large influence in the emergence, a genotype-environment interaction is suggested.

Our results indicate that under non-limiting moisture conditions, low temperatures can increase the emergence time, or even inhibit emergence, although the degree of such response may depend on the plant genotype. These observations may contribute in the design of management plans for the control of *Bromus* spp. To achieve a rapid and effective reduction of the weed on farms, a cropping system that facilitates uniform and rapid germination of *Bromus* before the low temperature period should be devised.

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POPULATION DYNAMICS OF *APERA SPICA-VENTI* AS INFLUENCED BY CULTURAL METHODS

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ABSTRACT

This paper deals with the effects of different cultural methods on the long-term development of *Apera spica-venti* populations in winter cereals, including the impact on crop yield. The effects of cereal crop (wheat versus rye), wheat cultivar and drilling date are simulated for two crop rotations using a difference equation model. The investigations showed that wheat cvs. with a short stem length strongly promoted the build up of *A. spica-venti* populations, as the seed production per plant is four to five times higher than cvs. with longer stems. Generally, population increases and yield losses were lowest with rye as the cereal crop due to its high competitiveness. Delaying the drilling date of winter cereals may reduce the rate of increase of *A. spica-venti* populations but it is stressed that this method might produce some undesirable side-effects. It is concluded that combining different cultural methods may reduce *A. spica-venti* infestations and so lessen the requirement for herbicide.

INTRODUCTION

In Denmark, *Apera spica-venti* has become more widespread in recent years because the area of winter cereals and winter rape has increased considerably. *A. spica-venti* can be satisfactorily controlled in most cases by several graminicides, but herbicide usage has become increasingly restricted and profit margins from arable cropping are declining. Consequently, researchers and advisers are looking for control strategies that require less herbicide use.

Cultural methods affecting *A. spica-venti* populations could be useful control measures in grass weed management systems. It is important to consider the possible long-term outcome of cultural methods, since cultural methods usually act with less efficiency than herbicides. Only a few experiments have been made to investigate the development of *A. spica-venti* populations in a long-term perspective (eg. Heitefuss et al., 1990; Hintzsche, 1993). However, population dynamic models may be another way to study the effects of specific cultural methods, provided that the parameters included have been quantified.

In this paper, the likely effects of a range of cultural methods on the long-term development of *A. spica-venti* populations in winter cereals, including the impact on crop yield, are simulated.

SIMULATION PROCEDURE

The cultural systems considered

The six cultural systems (1.-6.) mentioned below are simulated for two crop rotations (A and B), where ploughing is carried out every year:

A) Continuous cereal (wheat or rye).
B) Three years cereal (wheat or rye) followed by one year with a spring-sown broadleaved crop.

1. Early drilled (late September) winter wheat, cultivar with a short stem (60-80 cm).
2. Late drilled (mid-October) winter wheat, cultivar with a short stem.
3. Early drilled winter wheat, cultivar with a long stem (95-110 cm).
4. Late drilled winter wheat, cultivar with a long stem.
5. Early drilled (late September) winter rye.
6. Late drilled (mid-October) winter rye.

This gives 12 combinations, A1-A6 and B1-B6.

The simulation model

The simulations are based on a model consisting of two sub-models: 1) a demographic sub-model describing the life-cycle of *A. spica-venti*, and 2) a competition sub-model that describes the effect of *A. spica-venti* density on cereal grain yield. Mathematically, sub-model 1) is a difference equation incorporating a hyperbolic function that describes the density dependent relationship between plants m^{-2} and seed production m^{-2}:

$$S_{(t+1)}=S_t(1-e)(1-Al)+[SE(1-m)SP/(1+cSE(1-m))]SS \qquad (1)$$

S = seed population (numbers m^{-2}) in the soil at time of ploughing in the autumn in year t and the following year t+1
e = proportion of seeds giving rise to seedlings
Al = annual death rate of seeds in the soil
m = mortality rate of plants until seed shedding in July
SP = seed production per plant under conditions of no density stress
c = constant governing the response rate to increasing plant density
SS = survival rate of freshly shed seeds until ploughing in the autumn
SE = number of emerged seedlings in the crop estimated as:

$$SE=S_te \qquad (2)$$

The grain yield y as influenced by *A. spica-venti* density d, sub-model 2), is calculated from either of two equations. The yield of rye and wheat cvs. with long stems are calculated from a linear equation within a density range of 0-300 plants m^{-2} (Melander, 1992; Roder et al., 1985, 1986):

$$y=Y_{wf}-bd \qquad (3)$$

Y_{wf} = estimated weed-free crop yield
b = crop yield loss per weed plant

The rectangular hyperbolic model of Cousens (1985) is used in the case of wheat cvs. with short stems, since the relationship between grain yield and grass density typically is curvilinear (Melander, 1992):

$$y=Y_{wf}[1-id/(100(1+id/a))] \quad (4)$$

i = percentage yield loss per unit grass density as d approaches 0
a = percentage yield loss as d approaches infinity.

Parameter values

Parameter values are shown in Table 1. The known variation is shown in brackets. The values are obtained from literature and from field experiments finished recently. A more detailed description of these experiments will be published elsewhere in a companion paper. Here, results obtained from the experiments and from some minor investigations will be referred to as: (Melander, unpublished).

Parameter Al is taken from: Klemm (1990), Heitefuss et al. (1990), Jensen P.K. (personal communication). Parameter e, m, c and SS are from: Heitefuss et al. (1990) and Melander (unpublished). The competition parameters i, a and b are referred from: Melander (1992, unpublished), Roder et al. (1985, 1986). The stem length of the wheat cvs. is assumed only to affect the seed production SP and values are obtained from: Hagemeister & Heitefuss (1988), Franz et al. (1990), Heitefuss et al. (1990), Melander (unpublished). Drilling date is expected to affect parameter e, Y_{wf} and SP in rye whereas in wheat, e, Y_{wf} and the competition parameters i and a are expected to be affected (Melander, unpublished). It is assumed that seedling emergence does not occur in spring-sown crops. Normally, seedling emergence of *A. spica-venti* does not occur in spring-sown crops in Denmark.

The computation was limited to grass densities of 1-300 plants m^{-2}, because most parameter values have been estimated within that range.

RESULTS AND DISCUSSION

The simulation results are presented in Figure 1. The corresponding control level required in the cereals to prevent population increases is shown in Table 2.

It must be stressed that the simulations are based on means. Hence, the results must be considered solely as an example of what might be the quantitative outcome of practising the cultural systems. More information about the variation within the parameters is necessary in order to achieve an impression of the variation around the curves in Figure 1. Therefore, the qualitative indications as those shown in Table 2 are a fairer way of describing the general effects of the cultural systems on *A. spica-venti* populations.

With these reservations in mind, some general trends concerning cultural methods may be extracted. Rye impedes the development of *A. spica-venti* more than wheat because of its higher competitiveness. This is also seen on the impact on yield being relatively small in rye. In contrast, short wheat cvs. strongly promote the build up of *A. spica-venti* and yield losses become remarkably high when *A. spica-venti* densities increase. Long wheat cvs. act more like rye, although the competitiveness is not as high. The fact that *A. spica-venti* responds so strongly to crop height is a common experience in Denmark as well as in Germany (Pallutt B., personal communication). Both yield losses and seed production can be very high when panicles are emerging well above the crop (Franz et al., 1990; Melander, 1992, unpublished). In general, the stem length of cereals has been found to be positively correlated with the competitive ability against weeds (Christensen, 1992).

TABLE 1. Parameter values according to equation (1), (3) and (4). S is a short stemmed wheat cv. and H is a long stemmed one. ed and el are early and late drilling, respectively.

		e (%)	AI (%)	m (%)	SP (numbers)	c	SS (%)	i (% pl^{-1} m^{-2})	a (%)	b (kg m^{-2} pl^{-1})	Y_{wf} (t ha^{-1})
Wheat	**edS**	**2**	**80** (39-90)	**40**	**5449** (5183-5772)	**0.001**	**14**	**1.2** (0.2-2.2)	**74.8** (71.0-78.5)	-	**9.0**
	edH	**2**	**80** (39-90)	**40**	**1300** (635-1560)	**0.001**	**14**	-	-	**3** (1-6)	**9.0**
	ldS	**1.3**	**80** (39-90)	**40**	**5449** (5183-5772)	**0.001**	**14**	**1.0** (0.2-1.8)	**54.7** (30.9-78.5)	-	**8.7**
	ldH	**1.3**	**80** (39-90)	**40**	**1300** (635-1560)	**0.001**	**14**	-	-	**3** (1-6)	**8.7**
Rye	**ed**	**2**	**80** (39-90)	**40**	**865** (657-1073)	**0**	**14**	-	-	**2.2** (1-3)	**7.0**
	ld	**1.3**	**80** (39-90)	**40**	**609** (459-759)	**0**	**14**	-	-	**2.2** (1-3)	**6.8**
Spring-crops		**0**	**80** (39-90)	-	-	-	-	-	-	-	-

TABLE 2. The control level CL (%) required in the cereals to prevent *A. spica-venti* population increases, calculated for each cultural system shown in Figure 1. QI is a qualitative indication of the population increases and the associated control requirement following the cultural systems.

	A1	A2	A3	A4	A5	A6	B1	B2	B3	B4	B5	B6
CL	**92**	**87**	**64**	**44**	**45**	**0**	**84**	**75**	**31**	**0**	**0**	**0**
QI	**VH**	**H**	**M**	**M**	**M**	**L**	**H**	**H**	**L**	**L**	**L**	**L**

VH=very high H=high M=moderate L=low

Shifting the crop rotation towards more spring-sown broadleaved crops will generally reduce *A. spica-venti* infestations. This is not surprising, since in most cases seedling emergence does not take place in these crops.

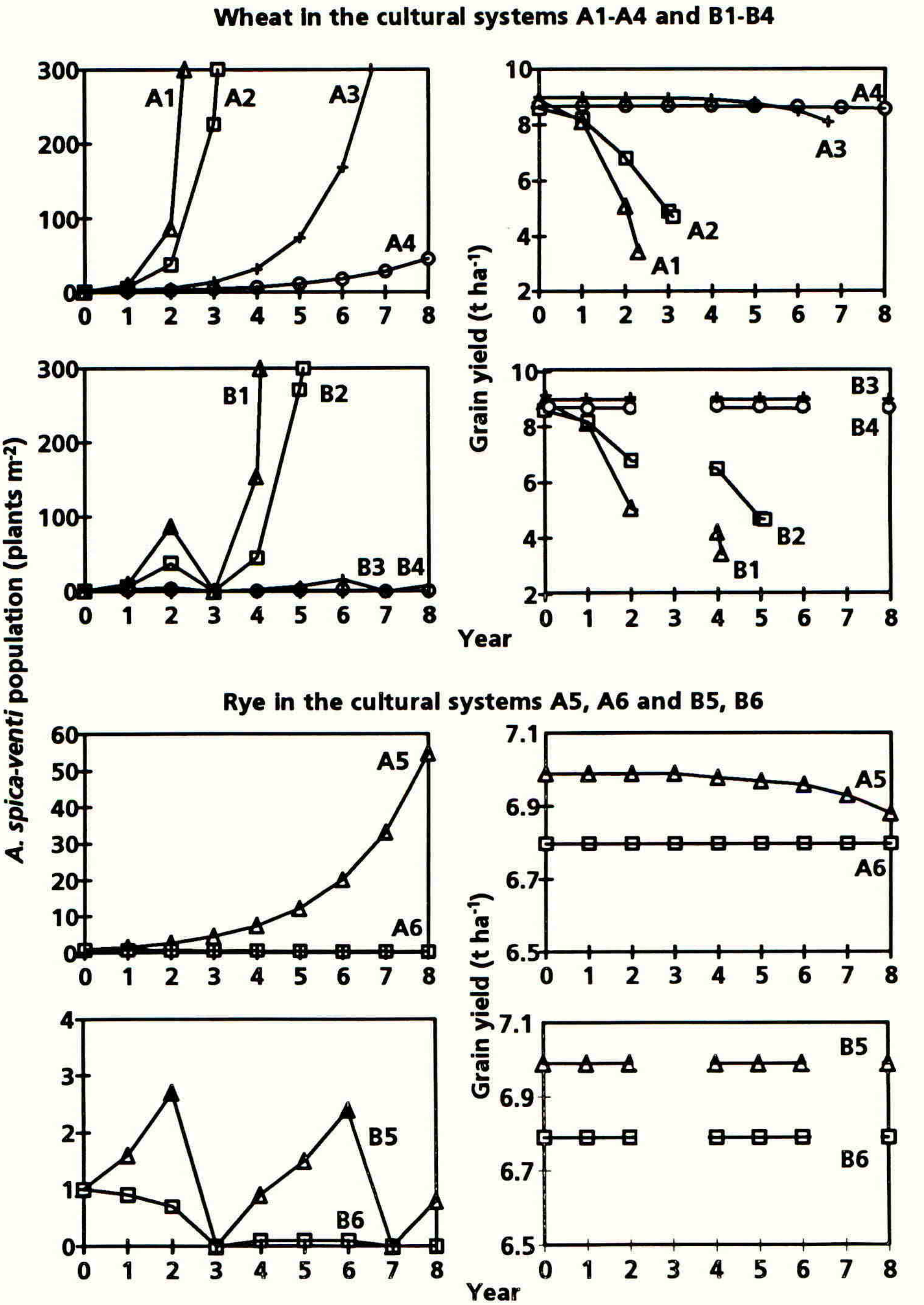

FIGURE 1. Left column: Simulated development of *A. spica-venti* populations in winter cereals as influenced by cultural methods. Starting density 1 plant m^{-2} in year 0. Right column: Corresponding effect on grain yield.

From Figure 1 it is seen that early drilling of winter cereals might cause higher population increases and yield losses than late drilling. However, drilling date has a much smaller effect than eg. wheat stem length and from practical experiences it is known that the magnitudes of the effects might change considerably from year to year. Furthermore, late drilling must always be weighted against the risk of a declined yield as well as the risk of adverse weather conditions increasingly impeding herbicide use. When considering these aspects, the advantages of delaying the drilling date appear to be very small.

Introducing cultural methods as well as cultivation methods into weed management systems as control measures against *A. spica-venti* undoubtedly will reduce the requirement for herbicide use, and probably make non-chemical control methods like weed harrowing more feasible. In conventional farming, however, one must realize that these methods will only be used if it is economically beneficial to the farmer or if he/she is otherwise forced to consider alternatives to herbicides.

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COMPETITION BETWEEN SPRING WHEAT AND *LOLIUM MULTIFLORUM* (ITALIAN RYE-GRASS)

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ABSTRACT

Lolium species including *Lolium multiflorum lam.* (Italian rye-grass) are common weeds of cereal fields in many countries. Two field experiments were conducted in 1991 and 1992 to investigate competition with spring wheat. In the first experiment a range of *L. multiflorum* sowing rates (0, 50, 100, 150 or 200 seeds m^{-2}) were planted into spring wheat, or grown on their own, and the effect on wheat yield and on the growth and reproduction of the weed was examined. The higher *L. multiflorum* populations resulted in a significant reduction in wheat growth. Wheat strongly decreased *L. multiflorum* dry weight, spike formation and seed production. In the second experiment a range of wheat densities (0, 25, 50, 100 or 200 plants m^{-2}) were sown with and without 100 plants m^{-2} of *L. multiflorum* to observe how crop population influenced weed growth. High wheat densities increased wheat yields with and without *L. multiflorum* present and gave very significant reduction in the growth and seed production of *L. multiflorum*. These results confirm the value of vigorous crops at competitive densities in suppressing this weed species.

INTRODUCTION

While Italian rye-grass (*L. multiflorum*) is a common grass crop, this, and other *Lolium* species, are also serious weeds of cereal fields in different parts of the world. Populations of *L. multiflorum* of 40 plants m^{-2} can result in up to a 30% loss in wheat yield (Appleby *et al.*, 1976). Severe competition from *L. multiflorum* can lead to fewer crop tillers, smaller heads formation and poor grain yield (Lemerle *et al.*, 1979). With effective herbicide treatments in four *L. multiflorum*-infested commercial fields in Oregon, wheat yields averaged 5.25 t ha^{-1} against 0.87 t ha^{-1} without control (Aldridge *et al.*, 1971). In a recent survey by one of the authors (K. S. Alshallash, unpublished) it was found that in an area of approximately 40,000 ha in the main cereal (wheat and barley) areas of Saudi Arabia, approximately 30% of farmers described annual *Lolium* species as serious weed problems and a further 40% as potentially serious ones. *Lolium rigidum* is the most common species present but *L. multiflorum*, *L. temulentum* and *L. persicum* are also found associated with *L. rigidum* in different areas. Grain yield losses in Saudi Arabia have not yet been quantified. The problem is increasing due to contaminated seed, frequent cereal crops in sequence, and the absence of effective control programmes. The effective use of herbicides was restricted by adverse conditions, poor application and the evolution of resistance. Therefore, the control of *Lolium* species should have high priority in any management system (Appleby *et al.*, 1976).

Over the last 20 years herbicides have been the most common method of *L. multiflorum* control in wheat. However, resistance to some herbicides has been reported (Moss *et al.*, 1993). Weed management strategies therefore need to be based on approaches wider than chemical control alone.

There are several cultural options for improving control of *Lolium* species control. The presence of vigorous crops at competitive densities has been a subject of research interest for several years. In Australia, effects of *Lolium rigidum* on crop yield were substantially reduced by increasing wheat density (Medd *et al.*, 1985). Barrett and Campbell (1973), in earlier glasshouse experiments showed that increasing densities of wheat decreased the effects of *Lolium rigidum* on competing mixtures. Increasing crop densities have potential relevance to suppressing weeds on the basis that growing crop plants exploit expanding volumes of 'biological space' (Harper, 1977). In Saudi Arabia wheat is usually sown at a low rate of 100 - 140 kg ha^{-1}. Annual weeds such as *Lolium* species can exploit the available space resulting from low wheat density, and this can result in the build up of large populations of grasses and poor grain yield. Thus both weed density and crop density need to be examined in relation to management practices. Two experiments were conducted, one in 1991 and one in 1992. The objective of the first experiment was to examine competition between a range of densities of *L. multiflorum* and a single density of wheat; the objective of the second experiment examined the effects of various densities of wheat grown with a single density of *L. multiflorum*.

MATERIALS AND METHODS

Both experiments were carried out in a field plot with an area of 240 m^2 at the University of Reading, U.K. The first experiment was sown rather late on April 9th 1991 and comprised a split plot design with eight replicates. Each main plot consisted of 5 pairs of subplots, each 3 m^2 area. Spring wheat (cv. Alexandria) was sown by hand at a rate of 100 seeds m^{-2} in one subplot of each pair and the other five subplots were uncropped. *L. multiflorum* (cv. RUP) was sown at five sowing rates (0, 50, 100, 150 and 200 seeds m^{-2}) on both the wheat and uncropped subplots and the seeds were lightly raked into the soil surface. No thinning was done in this experiment. N.P.K. (10:20:20) fertilizer (170 kg ha^{-1}) was given at sowing time and additional nitrogen was applied as ammonium nitrate at the three leaf stage to give 140 kg N ha^{-1}. Broad leaved weeds were controlled with MCPA and by supplementary hand weeding. The final harvest was taken by hand cut on 9th September on a 1.5 m^2 sample. *L. multiflorum* dry weight and the number of spikes were counted along with wheat dry weight and number of heads on the whole sample and grain weight/subplot calculated from a 50 head sample. Bulked samples of dry matter of straw and grain from wheat and straw of *L. multiflorum* were assayed for nitrogen content

The second experiment commenced on February 27th 1992. A randomised block design was used with eight replicates each consisting of 10 plots each of 2.25 m^2. Two plots were sown with each of the following rates of spring wheat (cv. Alexandria) 0, 35, 60, 110 or 210 seeds m^{-2}. *L. multiflorum* (cv. RUP) was sown at a single density of 110 seeds m^{-2} to one plot of each density of wheat. Populations of both wheat and *L. multiflorum* were thinned after emergence to give the required numbers of plants of wheat m^{-2} (0, 25, 50, 100, 200) and of *Lolium multiflorum* m^{-2} (100). Nitrogen was applied as ammonium nitrate at the three leaf stage to give 80 kg N ha^{-1}. The final harvest was taken on 3rd August by harvesting the whole plot. *L. multiflorum* dry weight, shoot numbers, spike numbers and spikelets number were counted. Wheat total and grain dry weights, tiller and head numbers were determined.

In both experiments wheat was sown in 200 mm rows while the *L. multiflorum* seed was broadcast. Data of both experiments were analysed using the S.A.S. programme.

RESULTS

Experiment 1

The effect of *L. multiflorum* competition on wheat

With sowing rates of 100 and 200 seeds m^{-2} of *L. multiflorum* there were significant decreases of up to about 25% ($P \leq 0.05$) both in wheat heads number m^{-2} and total dry matter g m^{-2} (Table 1). However wheat yields did not differ significantly ($P \geq 0.01$) between different *L. multiflorum* sowing rates and the N% of wheat dry matter was little affected by competition (data not shown).

TABLE 1. Effect of *L. multiflorum* sowing rates on wheat dry weight, grain yield and head densities.

L. multiflorum sowing rates m^{-2}	Total Dry Weight g m^{-2}	Yield g m^{-2}	Head numbers m^{-2}
0	1016.2	225.3	296
50	939.3	242.3	286
100	850.2	211.6	263
150	897.3	231.5	240
200	729.6	204.8	226
L.S.D. ($P \leq 0.05$)	155.8	67.0	46.2

Effect of wheat competition on *L. multiflorum* growth and reproduction.

L. multiflorum dry weight g m^{-2} was 8 - 21 times greater when grown without wheat competition (Table 2). Similarly, the presence of wheat resulted in a considerable reduction ($P \leq 0.01$) in *L. multiflorum* spike numbers m^{-2}. There was an increase in *L. multiflorum* dry weight and spike numbers with increased sowing rates when grown in the presence of wheat (Table 2) but a much smaller effect of sowing rate on both attribute when grown alone.

TABLE 2. Effect of wheat competition on *L. multiflorum* dry weight and spikes.

L. multiflorum sowing rates m^{-2}	*L. multiflorum* Dry Weight g m^{-2}		*L. multiflorum* spikes m^{-2}	
	- Wheat	+ Wheat	- Wheat	+ Wheat
50	714.0	33.8	90	29
100	793.2	64.0	98	42
150	786.2	70.1	90	45
200	835.1	103.0	90	61
L.S.D. ($P \leq 0.05$)	143.2		19	

Spikelet counts showed that there was an average of 16 spikelets/spike in *L. multiflorum* grown with wheat and 19 spikelets/spike in *L. multiflorum* grown without wheat. *L. multiflorum* dry matter tended to have a slightly higher per cent nitrogen content when grown with wheat but a smaller total nitrogen amount m^{-2} because of the very low dry weights m^{-2}.

Experiment 2.

Effect of wheat density on wheat yield in the presence or absence of *L. multiflorum.*

There was a trend for yields of dry matter and grain yields to increase with increasing wheat plant density. However, the grain yield were similar for the two highest densities, 100 and 200 plants m^{-2}. Heads m^{-2} changed progressively with plant density. There were substantial decreases relative to the weed free crop, in both total dry weight and grain yield when *L. multiflorum* was present, especially at densities of 50 or more plants m^{-2}. Increasing wheat density had beneficial effects when *L. multiflorum* was present such that yield with 100 wheat plants m^{-2} when the weed was present was similar to that of 50 plants m^{-2} when the weed was absent (Table 3).

TABLE 3. Effect of wheat density on wheat dry weight, grain yield and heads.

Wheat density plants m^{-2}	Total Dry Weight g m^{-2}		Yield g m^{-2}		Head numbers m^{-2}	
	*-Lm**	*+Lm*	*-Lm*	*+Lm*	*-Lm*	*+Lm*
25	753.1	740.7	434.7	376.6	258	233
50	961.4	768.7	562.6	405.4	334	251
100	1004.9	897.9	621.7	531.1	373	336
200	1174.5	952.0	624.3	549.1	437	361
L.S.D. ($P \leq 0.05$)	129.8		86		48.5	

* (*Lm* = *L. multiflorum*)

<u>Effect of the increase in wheat density in suppressing *L. multiflorum* growth and reproduction</u>.
There was a progressive decrease in all the measurements of *L. multiflorum* productivity (Table 4) as wheat density increased, with most differences between densities being significant. Even 25 wheat plants m^{-2} caused more than a 50% decrease in *L. multiflorum* dry weight but 50 plants m^{-2} were needed to decrease shoot, spike and spikelet numbers by this amount.

TABLE 4. Effect of wheat density on *L. multiflorum* dry weight, shoots, spikes and spikelets.

Wheat density plants m^{-2}	*L. multiflorum* Dry Weight g m^{-2}	Shoots m^{-2}	Spikes m^{-2}	Spikelets m^{-2}
0	521	549	547	9827
25	161	399	322	5464
50	109	276	224	4049
100	107	219	171	2880
200	59	127	109	1842
L.S.D. ($P \leq 0.05$)	70.4 (51.4*)	121.6 (88.2*)	117 (55*)	2501.7 (1434.2*)

* L.S.D. ($P \leq 0.05$) excluding wheat density of 0 m^{-2}

DISCUSSION

When sown without wheat *L. multiflorum* produced many tillers and spikelets especially when planted early. The presence of wheat however inhibited the growth and reproduction of *L. multiflorum* significantly, even with a high sowing rate of *L. multiflorum*. The reduction in *L. multiflorum* dry weight and spike numbers (Table 2) from wheat competition was evident over all *L. multiflorum* sowing rates, and gave substantial reductions in *L. multiflorum* spikelet production. These results confirmed that substantial *L. multiflorum* control can be achieved by the presence of vigorous crops. At high sowing rates of *L. multiflorum*, wheat dry weight was affected (Table 1). Appleby *et al.*, 1976, also reported significant grain yield losses with increasing *L. multiflorum* density.

Increasing wheat density up to 100 plants m^{-2} led to increased wheat yield (Table 3). Higher density of 200 plants m^{-2} did not increase crop yield further but did increase the suppressive effect on *L. multiflorum*. Wheat yield losses averaged about 17%. Medd *et al.*, (1985), reported *Lolium* suppression even at wheat density above which wheat yield was not increased by the high densities used. Our findings do not entirely support those of Medd *et al.*, 1985. In their model low densities of *L. rigidum* (up to 100 plants m^{-2}) with 40 wheat plants m^{-2} produced 100 times more biomass than that produced by the same *L. rigidum* densities with 200 wheat plants m^{-2}. In our experiment, there was only a 1.8 fold difference in *L. multiflorum* biomass between wheat populations of 50 and 200 plants m^{-2}. Clearly the interaction between weeds and crops vary considerably in different environmental conditions, and with different species.

Increasing crop density is an established means of decreasing the competitive effects of weeds such as *Avena* species (Cussans and Wilson, 1976) and other species (Zimdahl, 1980). The increase in wheat density resulted in a significant control of *Lolium multiflorum* growth and reproductive potential.

In Saudi Arabia low wheat seed rates are common (100 - 140 kg ha^{-1}). Such wheat densities would exert less competition on *Lolium* species allowing them to produce more seeds (Skorda and Efthimiadis, 1989). Large populations of *L. multiflorum* would be expected to develop unless they were controlled. Our recommendations agree with Skorda and Efthimiadis, (1989) that the use of seed rates of 180 to 210 kg ha^{-1} would provide better control of *Lolium* species, under semi-arid Mediterranean conditions and increase wheat yields. *L. multiflorum* is susceptible to an increase in wheat density and this could supplement the use of herbicides and contribute effectively to weed control. However, even with substantial suppression of seed production as noted in Table 4 there is still a large seed return so further studies on seed losses and seed survival in relation to soil management are still needed to improve control of such serious weed problems.

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Session 3B

The Effects of Chemicals on Non-Target Organisms

Session Organiser	Professor R H MARRS
Posters	3B-1 to 3B-9

HERBICIDES IN FARM FORESTRY: EFFECTS ON NON-TARGET INSECTS

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ABSTRACT

A series of experiments, in a joint venture between the Forestry Commission and MAFF, has been set up to investigate factors affecting tree establishment on ex-arable land. One such experiment, a study of the potential effects of root-feeding insects on establishing trees, used insecticides and herbicides to reduce levels of soil-dwelling insects and competition between weeds and young trees. A potential problem with the applications of pesticides is their effect on non-target species.

Here, experiments to assess the effect of two of the most commonly used herbicides in farm forestry, glyphosate and propyzamide, on the soil fauna are described. The larvae of the garden chafer, *Phyllopertha horticola* L., and adult carabid beetles, *Pterosticus melanarius* Ill., were used as models of insect species which are widespread in ex-arable land. The chafer is a generalist herbivore, feeding on both the roots of weeds and trees, while the carabid is an active generalist predator. The wider implications for insect community dynamics in the context of farm forestry are discussed.

INTRODUCTION

By planting trees on agricultural land, farmers can help reduce the current over-production of agricultural products, while bringing greater diversity to the countryside. To encourage the planting of trees on ex-agricultural land, the Government offers a number of schemes to help bridge the gap between planting and the first income from the thinning of the timber. Lowland areas with high fertility, relative to traditional forest sites, have the potential for a wide range of tree species to achieve high yields. However, there are factors associated with these sites which could hinder successful tree establishment. These include high pH, heavy soil textures and diverse weed and pest populations (Hibberd, 1988; Williamson, 1992). Experience gained by the Forestry Commission from early farm woodland experiments revealed that attention to initial weed control and subsequent vegetation management are keys to successful tree establishment on ex-agricultural land (Williamson, 1992). To investigate the factors affecting tree establishment on ex-arable land, a series of experiments, joint sponsored by the Forestry Commission and MAFF, has been set up. At Silwood Park, Berkshire, parallel experiments have permitted additional manipulations, including destructive sampling of trees.

The importance of effective weed control at the base of trees is well documented (Davies, 1987). However, it is generally not cost effective, or environmentally desirable, to maintain weed-free conditions over the whole site (Watson *et al.*, 1991). If good weed control is maintained around the base of trees, opportunities exist to establish ground cover between the weed-free areas. This practice also allows objectives such as game cover or habitat creation to be satisfied (Marshall & Nowakowski, 1991). Such habitat creation can form an integral part of farm landscapes and can interact with adjacent arable crops. For instance, many beneficial insects are associated with field margins and the enhanced populations of pest predators could play a role in integrated pest management.

The use of chemicals is the most popular method of weed control in establishing woodlands, with most of the UK's farm woodlands adopting this method. It is therefore important to consider the effect of any chemical applications on the soil fauna. Indeed, some herbicides are known to have insecticidal properties (*e.g.* Jess & Mowat, 1986; Croft, 1990). The experiments described aim to test the toxicity of two commonly-used herbicides on two dominant insect species, one herbivore and one predator. To make the tests as realistic as possible, different types of ground cover, typically found in farm forestry programmes, were employed. It was predicted that the effects of the chemicals may be more apparent on bare ground. As most of the chemical would be intercepted by a plant canopy, on bare ground where more chemical is available in the soil water, perhaps it is more likely to affect the abundance, diversity and functioning of the soil fauna (Brown & Lewis, 1990).

MATERIALS AND METHODS

Subterranean Insect Populations

The soil fauna was sampled from a farm woodland site at Silwood Park, Berkshire. Ten soil cores, each 10cm in diameter and 10cm in depth, giving a volume of 785cm^3, were taken from untreated areas and returned to the laboratory for hand sorting. Insects collected were preserved in alcohol and identified. To reveal the whole spectrum of organisms, soil sampling was repeated three times during the season, in April, July and October. Two commonly occurring insect species were selected as models representing different feeding types, to test the toxicity of two herbicides.

Herbicide Toxicity

Seed trays were lined with muslin and half-filled with moist compost (J. Arthur Bowers). Ten seed trays were used to represent each of three different ground covers. They were established as follows:

1. Bare Ground: each tray was topped with moist compost only.
2. Grass: turf was taken from a field at Silwood Park, abandoned from agriculture five years ago, and sited on top of the compost. This contained very few forb species.
3. Weed: turf was taken from a typical weed community developing on ex-arable land at Silwood Park and used as above. The turf contained a high number of annual and perennial forbs, but few grass species.

a) On an Insect Root Herbivore

To each tray, 20 final instar garden chafer larvae (*Phyllopertha horticola*) were added. (The larvae were collected from the field and stored at 5°C before use). The trays were stored for seven days in a glasshouse with a daily average temperature of 15°C and a minimum of 6°C. On the seventh day, five trays from each ground cover type were sprayed with a solution of glyphosate (Roundup, Monsanto plc.) at the recommended agricultural dose of 5 litres ha^{-1} (0.6kg AI ha^{-1}). The remaining five trays of each vegetation cover were sprayed with the equivalent volume of water. The trays were positioned randomly in a glass house and were kept moist by gentle watering for 35 days. After which, the trays were destructively harvested and the chafer larvae were recovered. It is known that chafer larvae can feed on organic matter in compost, so the bare ground treatment did not cause starvation.

The number of live and dead larvae were recorded, with any missing larvae being recorded as dead. The experiment was repeated using a spray solution of propyzamide (Kerb Flowable, Rohm and Haas (UK) Ltd.) at the recommended dose of 3.75 litres ha^{-1} (1.875 litres AI ha^{-1}).

b) On a Predatory Insect

To each of 10 trays, 10 adult carabid beetles (*Pterostichus melanarius*) were added. (The beetles had been collected from the field using pitfall traps and then stored at 5°C, before use). Propagator hoods were used to prevent escape of the beetles. In addition to the other soil fauna present in the two ground cover treatments, beetles were fed with pierced blow fly pupae (*Calliphora vomitoria* L.). The trays were stored as before, and on the seventh day, five trays from each ground cover type, were sprayed with a solution of Roundup, at concentrations of 5 litres ha^{-1}. The remaining five trays of each vegetation cover were sprayed with the equivalent volume of water. Trays were maintained as in a) for 35 days, when all beetles were removed and the number of alive and dead beetles was recorded, with any missing beetles being recorded as dead. As in a), the experiment was repeated using a spray solution of Kerb flowable, at the recommended dose of 3.75 litres ha^{-1}.

Analysis

The number of dead larvae or beetles were used as a measure of mortality and were analysed by a two-way analysis of variance, with treatment and ground cover as main effects.

RESULTS

Subterranean Insect Populations

A list of the invertebrates collected from the soil samples is given in Table 1 and illustrates the rich and varied communities of soil fauna in a farm woodland site. The numbers of soil invertebrates collected can be seen in Figure 1. The trend was for invertebrate numbers to fall in the summer due to the emergence of several taxa and increase again in autumn after oviposition. Numbers in excess of $100m^{-2}$ occurred during spring and autumn. Fifty percent of the soil fauna were root-feeding insects and seasonal density changes were the same as for other invertebrates.

TABLE 1 Invertebrates collected from the soil samples taken from a farm woodland site at Silwood Park, Berkshire. (* indicates most common taxa).

	ORDER	FAMILY	COMMON NAME	
Insects	Collembola	Onychiuridae	Springtails	
	Hemiptera	Aphididae	Aphids	
	Neuroptera	Ithonidae	Lacewings	
	Coleoptera	Carabidae	Ground beetles	*
		Curculionidae	Weevils	
		Elateridae	Wire worms	
		Scarabaeidae	Chafers	*
		Staphylinidae	Rove beetles	*
	Diptera	Bibionidae	March flies	
		Syrphidae	Hover flies	
		Tipulidae	Leatherjackets	*
	Lepidoptera	Hepialidae	Swift moths	
		Noctuidae	Cutworms	*
Centipedes				
Millipedes				
Spiders				*
Molluscs				
Earthworms				*

Herbicide Toxicity

Mortality of chafer larvae was less than 1% in most cases. Figure 2a shows that there was no significant difference between the mortality of chafers in the glyphosate treated or untreated trays ($F_{1,24}$=0.91, p>0.05), or between the type of vegetation cover ($F_{2,24}$=0.06, p>0.05), though there was a tendency for mortality to be higher in the two treatments with vegetative cover. Figure 2b shows the effect of propyzamide and, as with glyphosate, mortality of the larvae was less than 1% in both the treated and untreated trays. Again there was no significant difference between the mortality of chafers in treated or untreated trays ($F_{1,24}$=0.07, p>0.05), or was there any difference between the type of vegetation cover ($F_{2,24}$=0.87, p>0.05). There were no significant interaction effects between the herbicides and the ground cover on chafer mortality.

Similar results were found for the predatory beetles. Figure 3a shows there were no significant differences between beetle mortality of glyphosate treated and untreated trays ($F_{1,24}$=0.09, p>0.05), or between the type of vegetation cover ($F_{2,24}$=0.36, p>0.05). Similarly, Figure 3b shows propyzamide has no significant effect on beetle mortality ($F_{1,24}$=0.36, p>0.05), and that the results were unaffected by ground cover ($F_{2,24}$=0.27, p>0.05). As above, the herbicide treatments did not interact with the ground cover to affect beetle mortality.

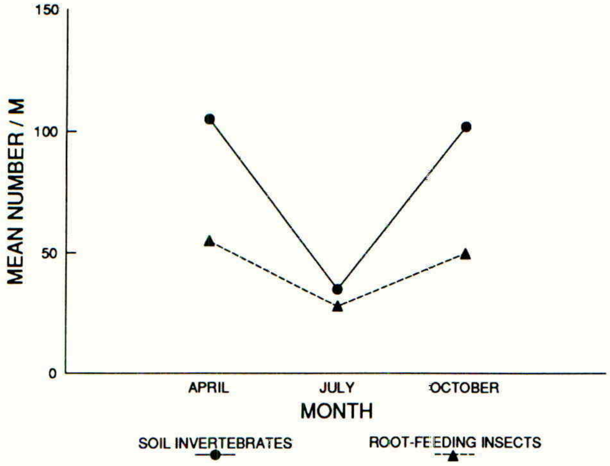

Figure 1 The numbers of soil invertebrates and root-feeding insects collected, using a soil corer, from a farm woodland site a Silwood Park, Berkshire.

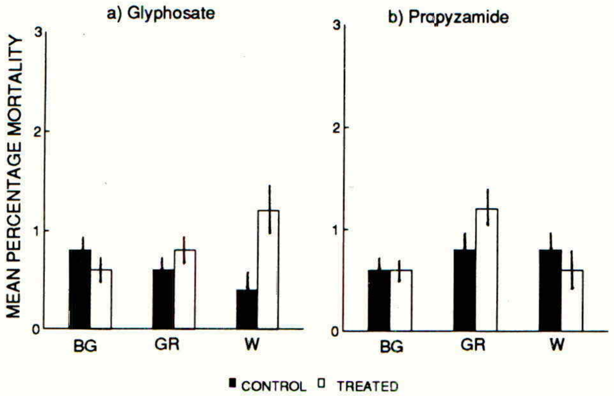

Figure 2 The effect of an application of a) glyphosate and b) propyzamide on the mortality of chafer larvae (*Phyllopertha horticola*). BG = bare ground; GR = grass; W = weed. Bars indicate +/- standard error.

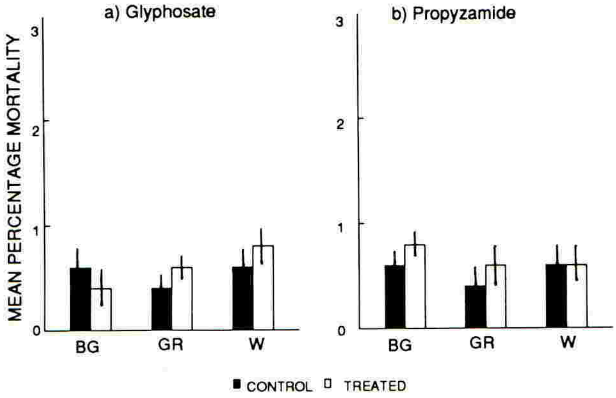

Figure 3 The effect of an application of a) glyphosate and b) propyzamide on the mortality of carabid beetles (*Pterostichus melanarius*). BG = bare ground; GR = grass; W = weed. Bars indicate +/- standard error.

DISCUSSION

The results of the soil sampling illustrate the rich and varied soil fauna that exists at the farm woodland site at Silwood Park. Sampling at other sites suggests this is typical. The most common insect families in terms of root herbivores were Scarabaeidae, Tipulidae and Noctuidae. In terms of insect predators, the Carabidae were by far the most abundant, while Staphylinidae were also common. The choice of insect species for the trials was therefore considered justified in representing two of the major insect taxa below-ground. In addition, they represented two of the main insect feeding guilds, herbivores and predators. The results indicated that glyphosate and propyzamide, two of the herbicides commonly used in farm forestry practice, caused no significant mortality of chafer larvae or adult carabid beetles. It was suggested that any toxic effect would be more apparent on bare ground as most foliar sprays are intercepted by the plant canopy. Surprisingly, the nature of the ground cover did not affect herbicide toxicity for either insect species. Thus, toxic effects of these herbicides can be considered negligible.

Other studies confirm these results, although there is insufficient data concerning the effects of propyzamide. However, while field experiments carried out by Eijsackers (1985) demonstrated that the effects of glyphosate on the soil fauna were negligible, some adverse effects were observed on a number of representatives of the soil fauna in laboratory studies. Such contradictory observations emphasise the need for tests to be undertaken in as natural conditions as possible, particularly in respect of ground cover. As well as glyphosate being non-toxic to soil-dwelling insects, it has been shown to have negligible effects on above-ground insects. A study on the cereal aphid, *Rhopalosiphum padi* L, which is important as a vector of barley yellow dwarf virus, showed that aphids were rapidly killed by both paraquat and diquat, but not by glyphosate (Wright *et al.*, 1985). Likewise, Zaitseva (1981) showed that applications of glyphosate did not affect the behaviour of the forest red ant (*Formica rufa*) or the expansion of ant hills.

Although there appears to be less information available concerning the toxicity of propyzamide on non-target organisms, the chemical is known to be non-toxic to fish (Orpin, pers. comm.). Also, the data collected by the IOBC/WPRS (Hassen *et al.*, 1987) showed that propyzamide was non-toxic to a number of insect natural enemies. The present study confirms this by comparing effects on a predator and a herbivore.

CONCLUSIONS

It therefore appears that the two herbicides, glyphosate and propyzamide, which are commonly used in farm forestry programmes are non-toxic, at least to the herbivorous and predatory insect species used here. It was impractical to test the herbicides on a full range of insect species that may be present in the field situation, so toxicity to specific organisms cannot be ruled out. However, it seems very unlikely that these commonly used herbicides would have any substantial effect on the non-target fauna. Insect communities would therefore be unaffected by the maintenance of weed free areas at the base of trees. Ground beetles are likely to increase in numbers in the vegetation maintained between the trees and therefore provide a useful control agent for adjacent arable crops. However, since the same will apply to soil-dwelling pest species there may be a need to protect the roots of trees since these will be readily eaten. This whole concept is developed elsewhere (Whitehouse, 1993).

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THE EFFECTS OF PRESTICIDES ON FOREST SPECIES. RESULTS OF A LONG-TERM GREENHOUSE TRIAL (1989-1993).

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ABSTRACT

A long-term greenhouse trial on forest species was started in 1989 to evaluate possible stress symptoms due to pesticides present in the environment. The hypothesis underlying this research is that small quantities of pesticides may concur in forest decline, altering plant physiological status. Two deciduous (*Carpinus betulus* and *Fagus sylvatica*) and two coniferous species (*Abies alba* and *Picea excelsa*) were tested. A range of non-destructive physiological parameters (transpiration, photosynthesis, stomatal conductance and chlorophyll) have been adopted to evaluate plant status. Results indicate changes in physiological parameters. Altered physiological status is not directly ascribable to the mechanism of action of pesticides but is indicative of more complex effects on plant metabolism.

INTRODUCTION

Pesticides may be an important factor in forest decline. In order to determine whether small amounts of AI affect forest species a long-term greenhouse trial on forest species was started in 1989 (Sicbaldi *et al.*, 1992). The hypothesis underlying this research is that small quantities of AI may concur in forest decline by altering plant physiological status. This paper reports data of a trial carried out to measure the effects on transpiration, photosynthesis, stomatal conductance and chlorophyll levels of pesticides on individuals of two deciduous (*Carpinus betulus* and *Fagus sylvatica*) and two coniferous species (*Abies alba* and *Picea excelsa*). These parameters reveal alterations in whole-plant status and are widely used as damage indicators in forest decline plants; these may therefore also be used as stress indicators (Lange *et al.*, 1989; Paoletti & Gellini, 1992; Evans *et al.*, 1993).

MATERIALS AND METHODS

One hundred and eighty individuals of two deciduous species (hornbeam, *Carpinus betulus*; beech, *Fagus sylvatica*) and 180 individuals of two coniferous species (silver fir, *Abies alba*; Norway spruce, *Picea excelsa*) were grown in a cold tunnel 15x3 m, oriented N-S, divided into 4 isolated chambers, 3 treated and 1 control. The plants were treated with a mixture of herbicides and insecticides (Table 1).

Treatments were twice monthly between May-September, with a total of 10 per year. On beech, silver fir and Norway spruce these were begun in 1990, on hornbeam in 1992. Pesticides are distributed using a overhead irrigation system which sprays deionized water at 13 litres/min directly on to the plants.

TABLE 1. Active components used in the mixture and total quantities distributed per chamber per treatment (μg). The mixture has been prepared using standards of AI used for analytical purposes.

Pesticide	chamber 1 minimum	chamber 2 medium	chamber 3 maximum
2,4-D	26	261	2611
Alachlor	26	260	2601
Atrazine	19	191	1914
Carbaryl	4	48	487
Diazinon	2	21	218
Dichlobenil	28	281	2819
MCPA	25	257	2579
Parathion	2	21	210
Phorate	3	30	306
Trifluralin	26	261	2611

The physiological parameters have been evaluated using non-destructive techniques: stomatal conductance (mmoles H_2O m^2/s), photosynthesis (μmoles CO_2 m^2/s), transpiration (mmoles H_2O m^2/s) and chlorophyll for deciduous species only (SPAD). The former 3 were measured using a portable CO_2 infrared analyzer ADC (Analytical Development Co., Hoddesdon, UK) in the following conditions; air sampler at 4 m from the ground; CO_2 flux set at 400 mg/l. Leaf chlorophyll content was measured using a Minolta SPAD-502; SPAD (Soil and Plant Analyzer Development) is also a unit proportional to the quantity of chlorophyll present in the area of leaf measured (4 mm^2). Five measurements per individual on up to 5 individuals per chamber have been taken to evaluate stomatal conductance, photosynthesis and transpiration. For coniferous species, 0-4 year-old branches have been measured separately. For deciduous species, 20 measurements per individual on up to 5 individuals per chamber have been taken to evaluate chlorophyll content. Coniferous species were analyzed in July and October 1992 and June 1993; deciduous species were analyzed in July 1992 (Evans *et al.*, 1993) and June 1993. Data were subjected to Analysis of Variance (ANOVA). The Tukey test (pairwise mean comparison) evaluates whether means for different groups are similar or not: (*) indicates significant differences compared to the control (P<0.05 or greater). Here a distinction between treatment and dose effect will be made: TREATMENT EFFECT indicates significant differences between the treated and untreated chambers only; DOSE EFFECT indicates a difference when response is a monotonic function of the dose.

RESULTS

Norway spruce (Table 2)

Photosynthesis

No difference in photosynthesis is reported for 1992 and 1993, although a dose effect trend is reported; June 1993 values were affected by high leaf temperatures.

TABLE 2. Norway spruce: photosynthesis (μmoles CO_2 m^2/s)

dose	July 1992	Tukey test	October 1992	Tukey test	June 1993	Tukey test
CONTROL	6.62	A	5.51	A	0.18	A-B
minimum	6.09	A	4.44	A	0.37	B
medium	5.25	A	4.84	A	0.59	A
maximum	3.98	A	7.40	A	0.15	A-B

Stomatal conductance (Table 3)

July 1992: TREATMENT EFFECT found, with values increasing (up to 200%) in all treated chambers. October 1992: DOSE EFFECT found, with the maximum dose (400% increase) different compared both to the control and the minimum dose; no difference between the control and the minimum dose. June 1993: no difference.

TABLE 3. Norway spruce: stomatal conductance (mmoles H_2O m^2/s)

dose	July 1992	Tukey test	October 1992	Tukey test	June 1993	Tukey test
CONTROL	78.25	B	108.57	A	14.82	A
minimum	189.07	A	73.43	A-B	17.92	A
medium	214.40	A	161.71	A-B-C	18.50	A
maximum	180.83	B	483.29	B-D	21.00	A

Transpiration (Table 4)

July 1992: TREATMENT EFFECT found, with values increasing up to 60%. October 1992: the maximum dose (80% increase) different compared both to the control and the minimum and medium doses. June 1993: no differences.

TABLE 4. Norway spruce: transpiration (mmoles H_2O m^2/s)

dose	July 1992	Tukey test	October 1992	Tukey test	June 1993	Tukey test
CONTROL	4.26	A	3.24	A	1.20	A
minimum	6.17	A-B	3.08	A	1.38	A
medium	4.22	B	3.94	B	1.44	A
maximum	6.96	B	6.20	A	1.61	A

Silver fir

Photosynthesis (Table 5)

No difference in photosynthesis is reported for 1992 and 1993.

TABLE 5. Silver fir: photosynthesis (μmoles CO_2 m^2/s)

dose	July 1992	Tukey test	October 1992	Tukey test	June 1993	Tukey test
CONTROL	0.93	A-B	1.89	A	4.48	A
minimum	1.91	B	1.60	A	7.47	A
medium	1.12	A-B	2.39	A	3.12	A
maximum	1.31	A-B	2.74	A	7.05	A

Stomatal conductance (Table 6)

July 1992: differences between the control and the maximum dose (200% increase); dose effect for branches of the years 1990÷1992, with an increase up to 300%. October 1992: difference between the control and the maximum dose (150% increase). June 1993: values are >1000 mmoles, because of excessively high leaf temperatures and are not considered significant.

TABLE 6. Silver fir: stomatal conductance (mmoles H_2O m^2/s)

dose	July 1992	Tukey test	October 1992	Tukey test	June 1993	Tukey test
CONTROL	12.52	A	29.93	A	478.30	A-B
minimum	27.95	B	23.00	A	1604.60	A-B
medium	17.80	A	27.55	A	250.10	B
maximum	28.15	B	47.88	B	1891.10	A-B

Transpiration (Table 7)

July 1992: possible TREATMENT EFFECT found: difference between the control and the maximum dose (100% increase); POSSIBLE DOSE EFFECT: difference between the control and the treatments (up to 110% increase), for the years 1989, 1991 and 1992 the treatments differ between each other. October 1992: TREATMENT EFFECT found: difference between the control and the maximum dose (50% increase); POSSIBLE DOSE EFFECT: difference between the control and the treatments (up to 90% increase), for the years 1990-1992. June 1993: TREATMENT EFFECT found: difference between the control and the maximum dose (70% increase); POSSIBLE DOSE EFFECT: difference between the control and the treatments (up to 90% increase), for the years 1991-1992.

TABLE 7. Silver fir: transpiration (mmoles H_2O m^2/s)

dose	July 1992	Tukey test	October 1992	Tukey test	June 1993	Tukey test
CONTROL	1.00	A	1.14	A	7.88	A-B
minimum	1.63	B	1.11	A	10.08	A-B
medium	1.25	A	1.15	A	7.42	A
maximum	2.00	C	1.62	B	12.96	B

Hornbeam (Table 8)

Stomatal conductance

TREATMENT EFFECT. The differences reported in 1992 are confirmed, with changes between 10÷25%. The difference between the control and the maximum dose is more marked in 1993.

Transpiration

Possible TREATMENT EFFECT. Difference between the control and chambers receiving the medium and maximum doses, with an decrease up to 20% is registered in 1993. Siginificant differences were not reported in 1992, but a trend is noticeable in the data.

Photosynthesis

1993 data dose not confirm the treatment effect (increase up to 200%) reported in 1992.

Chlorophyll

No difference in chlorophyll is reported for 1992 and 1993.

TABLE 8. Hornbeam: stomatal conductance (mmoles H_2O m^2/s); transpiration (mmoles H_2O m^2/s); photosynthesis (μmoles CO_2 m^2/s); chlorophyll (SPAD).

Dose	stomatal conductance 1992	stomatal conductance 1993	transpiration 1992	transpiration 1993	photosynthesis 1992	photosynthesis 1993	chlorophyll 1992	chlorophyll 1993
CONTROL	74.44	79.00	4.10	2.68	2.05	2.91	27.02	19.17
minimum	93.40*	73.37*	4.65	2.53	1.79	3.22	32.71	18.71
medium	101.70*	71.07*	4.98	2.40	3.79*	2.89	28.36	17.92
maximum	104.58*	62.50*	4.85	2.18*	4.19*	2.45	32.94	19.77

Beech (Table 9)

TABLE 9. Beech: stomatal conductance (mmoles H_2O m^2/s); transpiration (mmoles H_2O m^2/s); photosynthesis (μmoles CO_2 m^2/s); chlorophyll (SPAD).

Dose	stomatal conductance 1992	stomatal conductance 1993	transpiration 1992	transpiration 1993	photosynthesis 1992	photosynthesis 1993	chlorophyll 1992	chlorophyll 1993
CONTROL	126.32	78.37	5.73	3.34	5.45	4.07	35.65	29.56
minimum	135.56	112.50	5.95	4.19	4.17*	6.25*	28.02*	28.40
medium	117.85	90.12	5.37	3.43	4.81*	4.28*	25.18*	25.28*
maximum	128.54	80.75	5.67	3.47	6.77*	4.49*	26.73*	20.73*

Stomatal conductance and Transpiration

No difference reported either in 1992 or in 1993.

Photosynthesis

TREATMENT EFFECT. All treated chambers increase up to 250%. These effects are less marked than those registered in 1992, for which a DOSE EFFECT was evident.

Chlorophyll

DOSE EFFECT found. All treated chambers decrease up to 50%; this effect is more marked than in 1992, for which a treatment effect had been registered, with decreases up to 30%.

DISCUSSION

Treatments alter deciduous plant respiration and chlorophyll content and data are in agreement with those on damaged 'forest decline' plants (Evans *et al.*, 1993). In conifers plant respiration parameters were altered, without variation in photosynthetic activity. This would imply concomitant changes in epicuticular wax structure and stomatal response to treatments, rather than metabolic response resuling from plant stress.

Paoletti and Gellini (1992) indicate that in a range of physiological, morphological and biochemical parameters used to distinguish between healthy and damaged plants, stomatal conductance and transpiration appear to be the most sensitive: our data supports these conclusions.

CONCLUSIONS

It appears that physiological parameters used in this trial are not universally applicable, but rather that for each species the pertinent ones must be identified. Furthermore physiological parameters are not suitable for the development of response trends, since they are strongly influenced by atmospheric factors during sampling. However these physiological parameters provide overall indications on the effects of treatments; quantitative factors such as leaf/needle area, fresh and dry weight, wax weight, morphology and chemical composition, number and size of stomata will provide more reliable information. Doses used in this trial are similar to those registered in environmental samples (Trevisan *et al.*, 1993). Overall results indicate that registered physiological variations are ascribable to treatment with pesticides, indicative of possible metabolic disequilibria induced by the AI.

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EFFECTS OF HERBICIDES ON SOIL AND SURFACE-INHABITING INVERTEBRATES

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ABSTRACT

The extensive use of herbicides in the United States raises the issue of how they affect populations of soil and surface-inhabiting invertebrates and the important ecological processes that these organisms control. Soil and surface-inhabiting invertebrates are extremely important in influencing soil turnover, organic matter breakdown, nutrient cycling and the biological control of pests and diseases.

Herbicides can be directly, acutely or chronically toxic to soil-inhabiting invertebrates or can have indirect effects on them by changing the availability of organic matter in soil, the amount of soil cover, habitat and the availability of prey for beetles that prey on pests. Often their indirect effects can be greater than their direct ones.

This paper reviews the design and results of field experiments which identify the direct and indirect effects of cyanazine, benzoyl propethyl, atrazine and simazine on total arthropod populations, soil invertebrate biomass, earthworm numbers and biomass, and predatory beetle populations. The significance of these direct and indirect effects is reviewed.

INTRODUCTION

Much greater quantities of herbicides are used in the United States than those of any other pesticide, compared with Europe. This raises the issue of how these chemicals affect populations of soil-inhabiting invertebrates. These invertebrates are very important components of soil ecosystems. They play key roles in: maintaining soil fertility; as pests; or as natural enemies of pests. Earthworms (Lumbricidae) and soil-inhabiting arthropods, including mites (Acarina) and springtails (Collembola) and woodlice (Isopoda), millipedes (Diplopoda), and the larvae of many species of insects such as beetle and fly larvae, break down dead plant material in soil. They fragment this plant debris, thereby promoting microbial activity and releasing the plant nutrients that it contains. Many soil-inhabiting insects, including beetle, fly and moth larvae are important as pests of plants. Finally, arthropods such as centipedes (Chilopoda), predatory mites (Acarina) and some insects, particularly Carabidae and Staphylinidae, are important predators of soil-inhabiting pests.

such quadrats were sampled in each plot.

The effects of the herbicides on the activity of predatory surface-living carabid beetles was assessed by burying four, 10 cm diameter by 10 cm deep, pitfall traps containing glycerol in each plot. These were kept covered and opened up to collect beetles for a period of 48 hours at monthly intervals. Populations of these predatory beetles were also assessed by hand sorting the top 5 cm of soil in 0.5 m by 0.5 m quadrats. Four such quadrats were located in each plot.

In a second experiment, having the same experimental design but with plots measuring 5 m x 5 m, the herbicides: simazine (2 kg/ha) and atrazine (2 kg ha^{-1}) were applied in April and the effects on soil-inhabiting invertebrates compared with those in control and hand-weeded control plots. The experiment was cropped with winter wheat and the same assessments were made in this experiment as in the first experiment.

RESULTS

The effects of the herbicides on total populations of all arthropods are summarized in Figures 1 and 2. The effects of the herbicides on populations and biomass of earthworms are illustrated in Figure 3. The influences of the herbicides on the activity and numbers of predatory beetles are summarized in Figure 4.

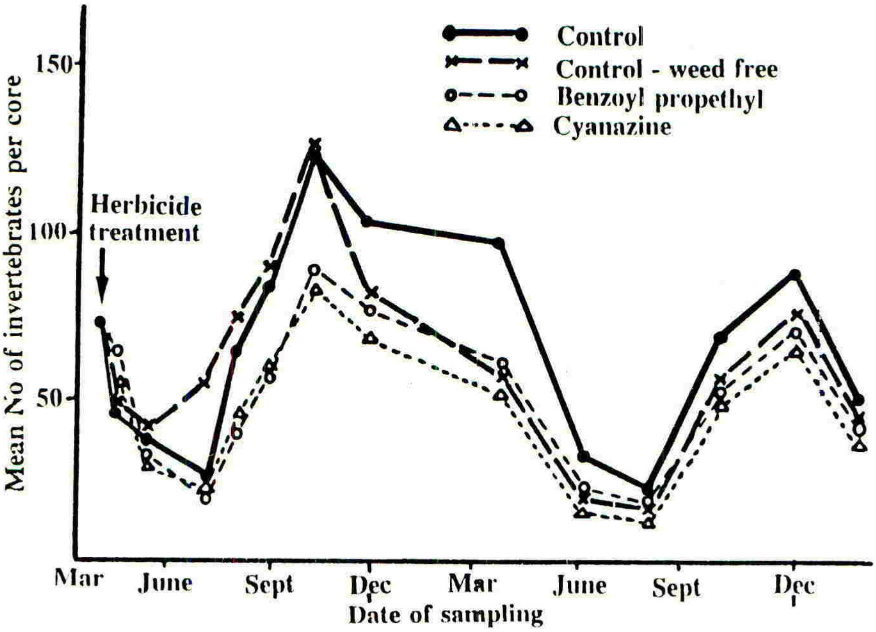

Figure 1. The effects of no herbicide, hand-weeding, benzoyl propethyl and cyanazine on populations of soil-inhabiting arthropods.

Herbicides can affect populations of soil-inhabiting invertebrates in different ways. They may: (i) be directly toxic to invertebrates; (ii) have chronic effects on invertebrate reproduction, activity, feeding or growth; (iii) act indirectly by changing the food supply of herbivorous or saprophagous species of invertebrates; (iv) influence invertebrate populations by killing predatory and parasitic arthropod species, which are often more susceptible to pesticides than other invertebrate species. The indirect effects of herbicides can often be much greater than the direct ones, particularly in the short term.

Assessing such complex effects of herbicide treatments on soil-inhabiting invertebrates in field experiments is difficult (Edwards and Stafford, 1979). The majority of researchers have used relatively simple replicated field trials and record only the overall effects of the chemical without identifying the route or mode of action of the particular herbicide (Edwards, 1989; 1991). Better knowledge of the direct toxicity of different herbicides to soil-inhabiting invertebrates, would allow a choice of herbicides with minimal side-effects on beneficial invertebrates to be made. The Working Group of the International Organization for Biological Control (I.O.B.C.) on 'Pesticides and Beneficial Organisms' has made considerable steps in the assessment of direct toxicity to beneficial invertebrates, using laboratory or semi-field tests (Pisl, 1988; Mazzone and Viggiani, 1988; Hassan *et al.*, 1991), but we still need well-designed field experiments to support such results.

In this paper I describe the design of field experiments and methods of sampling for invertebrate populations that emphasize the assessment of direct effects and minimize the indirect effects of herbicides.

EXPERIMENTAL METHODS

In a randomized block design experiment, with four replicates of four treatments, one set of 10 m x 4 m plots was sprayed with benzoyl propethyl (1.5 kg/ha) and another with cyanazine (2.0 kg/ha) in April. The experiment was cropped with winter wheat. Two sets of replicates were left unsprayed. One of these two sets was allowed to grow weeds and the other was maintained with no weeds by hand-weeding.

To assess the effects of the herbicides on arthropod populations, four soil cores 5 cm diameter and 15 cm deep were taken from each plot at one to two-monthly intervals. The invertebrates in these samples were extracted into 70% ethyl alcohol, in modified high-gradient Tullgren funnels and identified to genus, order or functional group.

Earthworm populations were sampled twice annually in spring and autumn. To do this, nine litres of water containing 50 cc of 40% formalin were poured on to a quadrat measuring 0.5 m x 0.5 m. The earthworms which were brought to the soil surface, were collected and preserved in 5% formalin, until they could be identified, and weighed after soaking up surplus formalin on a filter paper or paper towel. Four

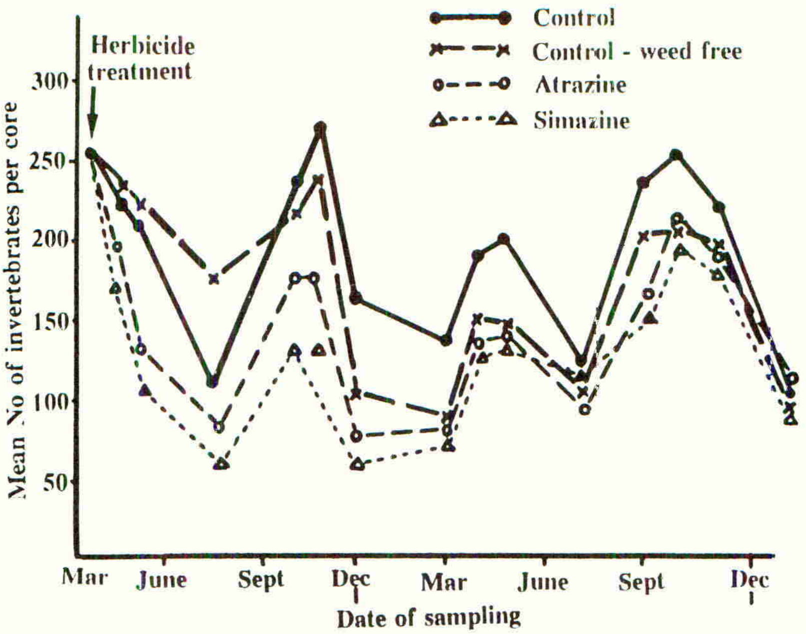

Figure 2. The effects of no herbicide, hand-weeding, atrazine and simazine on populations of soil-inhabiting arthropods.

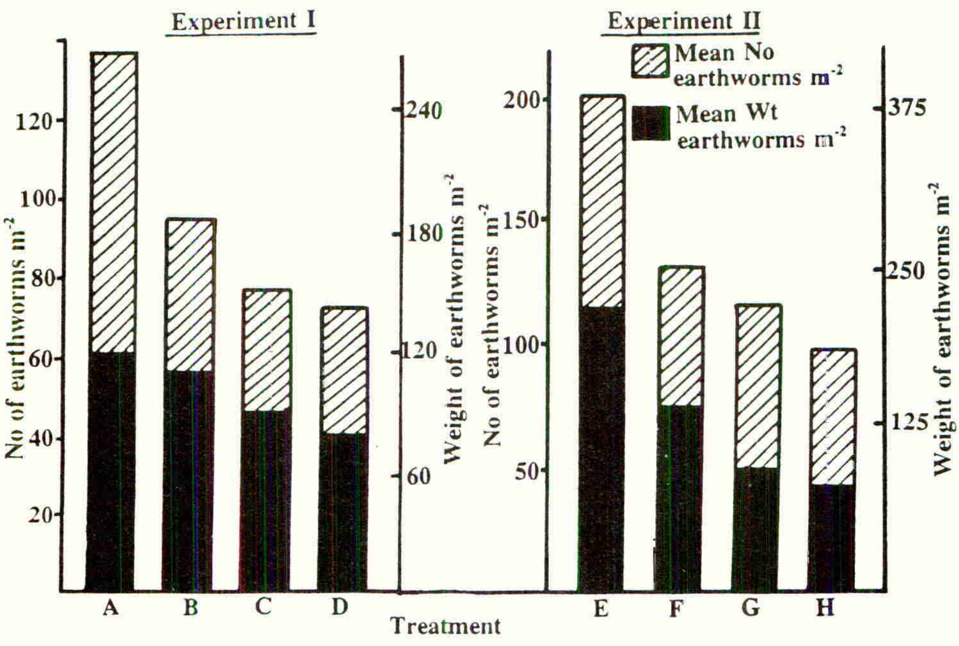

Figure 3. The effects of no herbicide (A & E), hand-weeding (B & F), benzoyl propethyl (C), cyanazine (D), atrazine (G) and simazine (H) on earthworm populations.

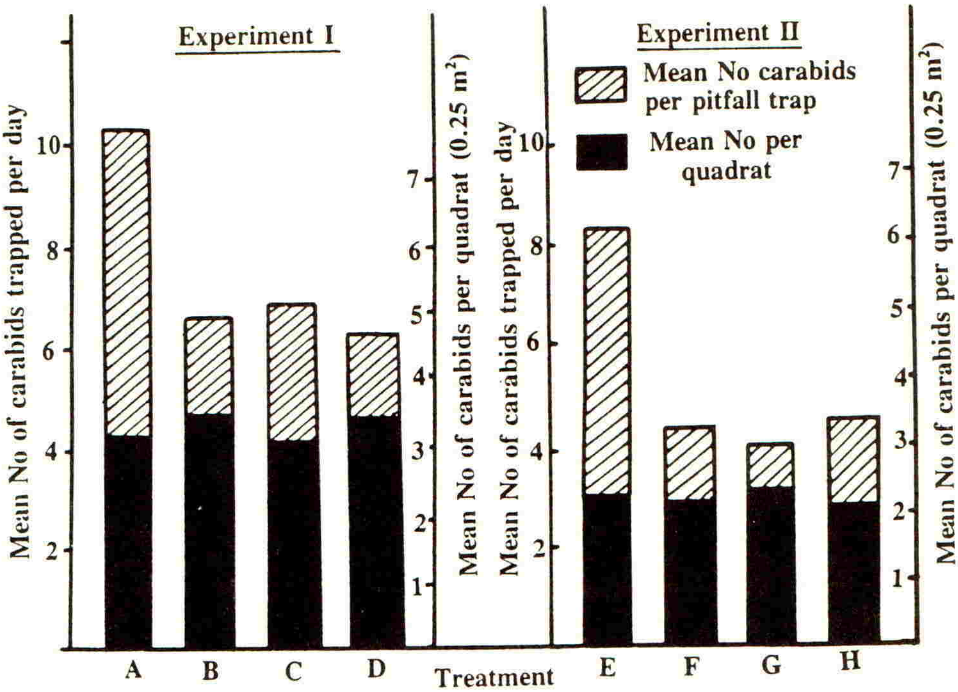

Figure 4. The effects of no herbicide (A & E), hand-weeding (B & F), benzoyl propethyl (C), cyanazine (D), atrazine (G) and simazine (H) on carabid beetle population.

DISCUSSION

Most studies of the effects of herbicides on soil-inhabiting invertebrates have concluded that the majority of herbicides have relatively small overall effects (Edwards, 1989). However, most of these field experiments did not distinguish between the overall effects of the herbicide on soil invertebrate populations, and the direct toxicity of the chemical to invertebrates. The indirect effects of the herbicide decreases surface plant cover and provides a pulse of input of decaying plant material into soil, in the form of dead weeds. Both of these indirect effects can have a significant effect on populations of many soil-inhabiting invertebrates; indeed, these are usually greater than those due to the direct toxicity of the herbicides (Edwards, 1989).

The field experiments described here, compare the overall effects of four herbicides, on numbers of soil-inhabiting arthropods, surface-living carabid beetles and earthworms, with the effects of applying no herbicide (control) and removing weeds by hand-weeding, on these invertebrates. This makes it possible to assess any direct chronic and acute toxic effects on the soil fauna much better as well as to assess the overall effects of the herbicide treatment.

Clearly, hand-weeding and allowing these weeds to die on the soil surface caused quite distinct, but relatively short-term, increases in populations of soil-inhabiting arthropods followed by a large period of decreased numbers (Figure 1). Nevertheless, all of the four herbicides tested reduced populations of these invertebrates even further than hand-weeding i.e. there is evidence of some direct toxicity to soil-inhabiting invertebrates for all of the four chemicals that were tested in the field.

Similarly, hand-weeding tended to decrease overall populations of earthworms, presumably by decreasing the availability of decaying organic matter derived from weeds in the soil, and all of the four herbicides decreased earthworm populations even further than hand-weeding (Figure 2).

The effects of the herbicide and hand-weeding treatments on the surface-living carabid beetles was much more complex. This was demonstrated clearly by comparing the results from the two methods used to sample the carabid beetle populations. Catches in pitfall traps assess not only carabid beetle populations but also their activity. There is considerable evidence that carabid beetles are much more active when ground cover, such as that provided by weeds, is available. Hence, although the overall effect of all of the four herbicides tested on pitfall trap catches of carabid beetles was quite large, when the data are compared with those from direct population estimates based on quadrat searches, it becomes clear that none of the four herbicides had much direct toxicity to carabid beetles (Figure 3). These experiments, in addition to confirming that these four herbicides are moderately toxic to soil-inhabiting arthropods and earthworms, and have a considerable indirect effect on them, demonstrate the need for careful design of field experiments in assessing the overall effects of herbicides and other pesticides.

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SELECTION OF RECEPTORS FOR MEASURING SPRAY DRIFT DEPOSITION AND COMPARISON WITH BIOASSAYS WITH SPECIAL REFERENCE TO THE SHELTER EFFECT OF HEDGES

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ABSTRACT

Five types of receptors are compared for measuring spray drift deposition using fluorescein as a tracer. They were all found to have similar efficiency over a distance of 32 m downwind of a hydraulic sprayer. Plastic hair curlers were chosen to measure the shelter effect of hedges by comparison with drift through gaps in the hedge. These results are shown to be closely matched by a bioassay where damage from MCPA drift was assessed using seedlings of Ragged-robin (*Lychnis flos-cuculi*). The combined use of deposition measurements and bioassays is discussed.

INTRODUCTION

Many studies on pesticide spray drift have shown an exponential decline in drift deposition with distance downwind from the sprayed area when measured over uniform vegetation such as short grass. However, such results do not measure effects directly on non-target species, which do not necessarily show a similar pattern of response. The species may show no effect at any distance if it is not susceptible to a particular compound; or it may suffer 100% response for some considerable distance before the effect declines with further distance. Unfortunately, few studies have combined deposition studies with bioassays, mainly because of the difficulty of measuring most pesticides directly at the low levels found in drift. Figure 1 gives an example of a bioassay combined with a crude estimate of drift determined using water-sensitive papers. In this case, drift deposition of diflubenzuron declined to less than 1% of the deposition at the edge of a sprayed area while the mortality of caterpillars feeding on horse radish (*Armoracia rusticana*) remained at 100%. Fifty per-cent mortality occurred at about 8 m downwind where visible deposition had declined to about 0.1%. This discrepancy is partly due to the fact that the smallest droplets will not impact on these flat targets, but there is no doubt that bioassays can provide very sensitive measures of drift.

Most studies on drift deposition have used a fluorescent dye or other tracer which can be measured by a physico-chemical assay technique. The receptor targets themselves range from broad flat plates, generally placed horizontally, to long lengths of thin polythene tubing or nylon yarn oriented horizontally or vertically (Gilbert & Bell, 1988; Western & Hislop, 1991).

The latter are much more 'efficient' collectors of the small droplets which are likely to be deposited on narrow plant stems and insects. Other supposedly efficient targets that have been used include pipe cleaners

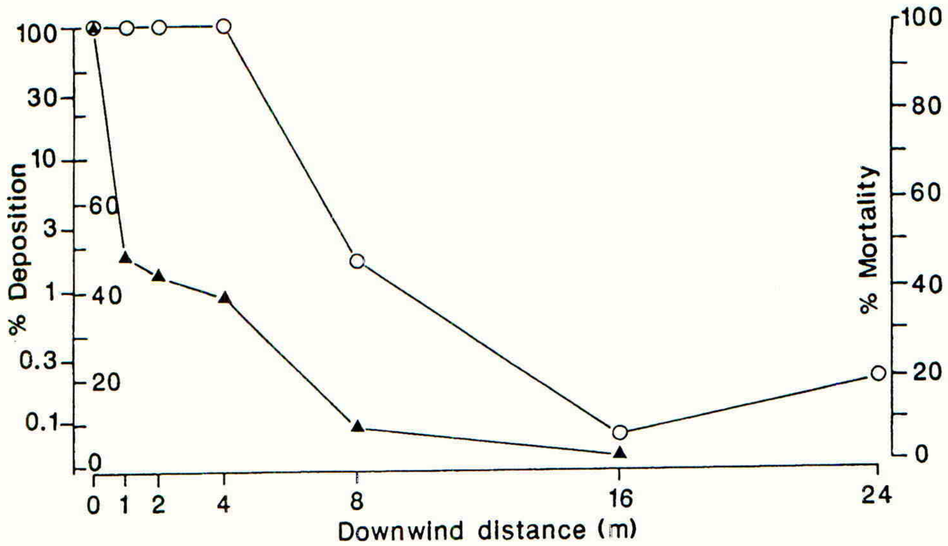

Figure 1 Spray drift deposition of diflubenzuron (triangles) and resulting mortality of *Pieris brassicae* larvae (circles). Deposition as percentage of that at edge of sprayed area plotted on logarithmic scale. (Adapted from Davis *et al* 1991)

(May, 1991; Nordbo & Taylor, 1991) test tube brushes (Cross, 1991) and hair curlers (Parkin & Merritt, 1988). Such small, discrete targets can be used to obtain detailed profiles of drift deposition in and around vegetation to help with the interpretation of bioassay results when wind turbulence is likely to distort simple relationships with downwind distance.

This paper describes the selection of a suitable target for studies on the shelter effect of hedges and compares drift deposition with a bioassay using a native plant.

METHODS

Choice of receptor

Five types of receptor target were compared:

- Plastic rods 1.6 mm diameter x 180 mm long,
- Pipe cleaners 3 mm x 180 mm,
- Test tube brushes 10 mm x 100 mm,
- Plastic hair curlers 38 mm x 60 mm ('Cut-A-Roller'),
- Cylinders of aluminium mesh 30 mm x 80 mm (made from 'David's Car Repair' sheets).

These targets were suspended from stakes with cross bars 45 cm above ground. Three parallel rows of stakes were set out 10 m apart at 1, 2, 4,

8, 16 and 32 m downwind of the area to be sprayed in a field of short grass. Sodium fluorescein was used at 350 mg/1 in the spray tank. Spraying was carried out using a 6 m boom 0.75 m above the ground with 12 flat fan nozzles (Lurmark Red 03-F80) operating at 2 bar pressure giving an application rate of 150 l/ha and VMD of 255 µm. Wind speed during the trial was 2.0 m/s at 1.5 m height;
relative humidity was 77%.

Deposits were extracted with sodium hydroxide solution (0.02M) and a surfactant (0.01% 'Agral'), and the concentrations measured using a Shimadzu RF5001PC spectrofluorophotometer. By altering the sensitivity range, dye concentrations in the range 0.001-1 mg/l could be measured. Results were corrected for recovery and fade measured in spiked samples (see Brown, 1991).

Drift experiments over hedgerows

A series of three spray drift experiments was done using the chosen receptors to compare the profiles of drift deposition over a thick hawthorn hedge (1.6 m high x 1.2 m wide) with those through wide gaps in the hedge. Spraying was carried out as described above 6 m upwind of the hedge, and the receptors were placed at three heights (0.45, 1.0 and 2.0 m above ground) at distances between 1-20 m downwind of the sprayed area, ie up to 13 m downwind of the hedge (or gap). A bioassay trial was subsequently carried out using MCPA and Ragged-robbin (*Lychnis flos-cuculi*) seedlings. Approximately 600-700 plants were placed in seed trays on the ground at 1, 5, 7 and 15 m downwind of the sprayed area and damage assessed after one week.

RESULTS

Comparison of spray drift receptors

All receptors showed similar exponential declines in fluorescein deposition with distance downwind of the sprayer, though the mean deposits at any one distance varied by a factor of 5-10 between the five types. The highest values were obtained with the two large perforated cylinders, the plastic hair curler and the aluminium mesh, the lowest with the plastic rods. When the results are plotted on log - log axes the declines in drift deposition are seen to follow more or less parallel lines in all cases, with especially close correlation between the hair curler and mesh cylinder (Figure 2).

These results indicated that the five types of receptors were all of similar efficiency over the range of droplet sizes encountered in this trial. The pipe cleaners appeared to be rather more efficient than the test tube brushes at greater distances. The means of the standard errors, when calculated as percentages of the measured deposition at each distance, were lowest for the cylinders (10.2%) and rods (11.4%) and highest for the test tube brushes (16.1%).

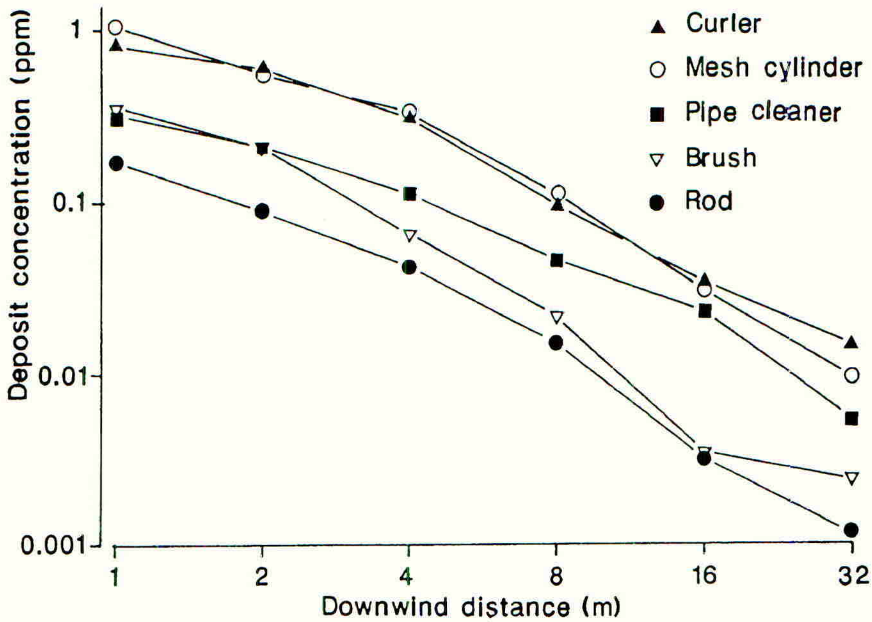

Figure 2 Drift deposition of fluorescein measured by five types of receptors

The plastic hair curlers were chosen for subsequent studies on spray drift because the alkaline washing solution reacted with the aluminium receptors causing gradual corrosion.

Hedgerow studies

The three spray drift trials with hedges and gaps all showed a pronounced shelter effect immediately behind the hedge for targets placed below hedge height; at 7 m downwind of the sprayed area, the depositions on targets 0.45 m above ground were 10-25 times greater in the gaps than behind the hedge for wind speeds of 2.0-3.0 m/s. However, this shelter effect was reduced further downwind until there was little or no difference between deposition values 13 m behind the hedge or gap. Figure 3 shows the mean results for the three trials plotted on a logarithmic scale as percentages of that at 1 m.

The bioassay with *Lychnis flos-cuculi* likewise showed a marked shelter effect immediately behind the hedge but increased damage from the MCPA spray 8 m downwind of the hedge. The bioassay trial was done some time after the fluorescein trials and under stronger wind conditions so direct comparisons cannot be made. Nevertheless, there was clearly a

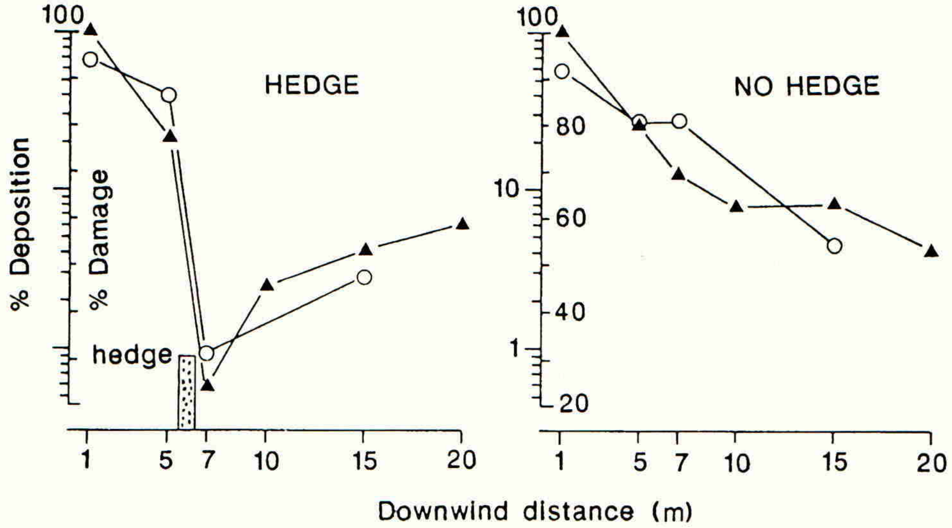

Figure 3 The effect of a hedge on drift deposition (fluorescein shown as % of deposition at 1 m, triangles) and on damage to *Flos-cuculi* seedlings from MCPA spray drift (circles)

general similarity between the profiles of severe damage and drift deposition. This similarity is evident in Figure 3 where the percentages of severely damaged plants are plotted on an arithmetic basis beside the deposition curves at an appropriate scale. Full details of these hedgerow studies are given in Davis *et al.* (in press).

DISCUSSION

Experiments are now in hand to combine the use of a tracer such as fluorescein with a herbicide or insecticide so that drift deposition and effect can be related directly. This approach would also be useful in applied field studies where validation of the level of pesticide application is often needed before conclusions on spray drift can be made with full confidence. Unfortunately, fluorescein cannot be used on consumable crops. Although it decays rapidly in sunlight and is not known to have any harmful effects on the environment or human health, it has not been rigorously tested and approved for such uses. In such circumstances it may be possible to use a foliar nutrient containing manganese or molybdenum which is compatible in a tank mix with the required pesticide, is not harmful to the test species, and can be measured by atomic absorption spectrometry. Fluorescein may have uses in other situations, for example in assessing drift of asulam in bracken control (cf Marrs *et al*, 1992).

The choice of receptors for measuring spray drift will depend on particular needs. The hair curlers proved satisfactory in the hedgerow studies though their complex geometry does not allow exact description or modelling. The aluminium mesh cylinders are simpler and would have advantages over plastic receptors where organic solvents were needed to extract pesticide deposits themselves.

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GUIDELINES FOR TESTING EFFECTS OF PESTICIDES ON NON-TARGET PLANTS

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ABSTRACT

In November 1992 representatives from regulatory authorities, research organisations and industry met to discuss testing for effects of pesticides on non-target plants. Different approaches are used in testing and risk assessment in USA, Canada and the UK. The USA has had guidelines in place for some time, whereas Canada and Europe are currently developing their guidelines. This process provides an opportunity to develop an internationally harmonised approach, using appropriate tests and species to ensure cost-effective of environmental protection. Some ideas are put forward for a testing scheme.

INTRODUCTION

At the SETAC (Society of Environmental Toxicology and Chemistry) meeting in Cincinnati in November 1992 a session on plant testing was held with representatives from regulatory authorities, the pesticide industry and research workers (Peterson *et al*, 1993a). Presentations were given on testing methods, risk assessment, and on effects measured on non-target plants in the field. This was followed by an open discussion session on testing the effects of pesticides on non-target plants. The need for this session had become evident as the lack of internationally agreed guidelines was a major impediment to cost-effective protection of non-target plants. Where data existed for non-target plants it had often been collected using too few species resulting in poor risk assessments (Peterson *et al*, 1993b).

The US Environmental Protection Agency published guidelines on plant testing in 1982 in Subdivision J of the Pesticide Assessment Guidelines. An evaluation of these guidelines (Fletcher and Ratsch, 1991) suggested that there should be harmonisation of the differences in test procedures between regulatory authorities and a move towards adopting universal standard tests throughout the international community. In Canada guidelines have been proposed for testing the effects of chemical pesticides on non-target plants (Boutin, Freemark and Keddy, 1993); these were presented at the session in Cincinnati. Although the UK has recently included non-target aquatic plants in its risk assessment for herbicides and plant growth regulators, it otherwise has little in the way of formal plant testing guidelines

(Aldridge, 1993). However, some research has been done in the UK on the effects of spray drift on non-target plants, with a view to setting buffer zones around semi-natural habitats (Marrs *et al*, 1993). Development of a European risk assessment scheme is currently underway through the Council of Europe and EPPO.

COMPARISONS OF APPROACH TO NON-TARGET PLANT TESTING

Harmonisation of regulatory requirements is desirable to reduce the costs to industry of data generation. However, individual regulatory authorities will each defend their own set of guidelines, which they consider to represent the best approach. There is a need to examine both the similarities and differences between guidelines, then to find a way to resolve the differences based on the findings of current research. Table 1 compares the approaches used by the USA, Canada and the UK.

TABLE 1: Approaches to regulatory testing in USA, Canada and UK

USA	CANADA	UK
• Test phytotoxic pesticides used on non-food crops.	• Test all chemical pesticides used outdoors.	• Test herbicides used outdoors.
• Tier I single dose tests on 1-4 spp algae, *Lemna gibba*, + 6 dicot, 4 monocot species.	• Tier I single dose tests on 3-6 spp of algae, 10-30 spp of vascular plant (screening data).	• Tier I EC50 tests on one species of algae and *Lemna* spp.
• Tier II if ≥50% effect on algae or ≥25% other species. 5 doses to include Tier I species.	• Tier II tests ≥50% effect on algae or ≥25% on other species. 5 doses (screening data).	• Tier II tests if ≥50% effect at label rate. May be laboratory, mesocosm or field studies.
• Tier III field testing if >25% effect, or 50% in aquatic species.	• Tier III test additional species of aquatic plants if ≥50% effect algae/*Lemna*. • Tier IV multispecies /community tests requested case-by-case.	
• EEC based on water depth of 15 cm; soil distributionto 3 cm, 60% overspray; 5% spray drift deposition.	• EEC based on water depth of 15 cm; soil distribution to 3 cm; 100% overspray; 10% spray drift deposition.	• EEC based on water depth of 100 cm; 100% overspray; 0.7% spray drift deposition.
• No uncertainty factor.	• Uncertainty factor of 10.	• No uncertainty factor.

Pesticides to be tested

The first step in the EPA scheme is to determine whether a chemical is phytotoxic, if so Tier I testing is required, all herbicides are tested first at Tier II. No herbicide phytotoxicity data are required for application solely to food/feed crops by ground equipment, if the volatility is less than 1.0 x 10^{-5} mm Hg and solubility less than 10 mg/l. Exceptions to this are where there is documented evidence of adverse effects in the field, potential for adverse effects to endangered species, or a Special Review (Lewis and Petrie, 1991).

This contrasts with the approach in Canada where testing for effects on non-target plants is required for all chemical pesticides used outdoors (Boutin, Freemark and Keddy, 1993). However, registrants may be granted a waiver for any test required if justified on appropriate scientific grounds. The UK has limited requirements for testing, with data only requested on the effects of herbicides and plant growth regulators on aquatic plants. In recent years, where a herbicide is to be used in non-crop areas that are not usually sprayed, eg bracken control, the effects on the non-target native flora have been considered. However, there are no instances of a full risk assessment being made for recent registrations of such uses, manufacturers opting not to pursue the recommendation instead (Aldridge, 1993).

Pesticides other than herbicides have been shown to have a detrimental effect on non-target plants (Swanson *et al*, 1991), as similarly it is not only insecticides that have adverse effects on beneficial arthropods (Brust, 1990, Sotherton, Moreley & Langley, 1987). Moreover, non-target native plants of conservation interest may occur in areas of semi-natural habitat adjacent to or in small pockets and corridors amongst agricultural land, and these plants may be worthy of protection. Therefore in any efforts towards harmonisation it is reasonable to require testing for all chemical pesticides used outdoors for their effects on non-target terrestrial and aquatic plants, unless it can be shown that exposure will not occur.

Tier I and II testing

Tier I testing differs between regulatory authorities in the number of species tested and which species these should be (Table 1). The UK has the lowest testing requirement, ie one species of alga and *Lemna*, spp. The US EPA has similar aquatic plant testing requirements, although with up to four species of algae being tested. Their recommended terrestrial plant species are based entirely on crop plants. Use of crop plants has an advantage in terms of laboratory testing in that more consistent results can be obtained. However, crop plants may not be representative of the species to be protected, nor of the variability within the wild flora. For Canada registrants are required to submit all plant screening data generated during product development. Single dose data are required for Tier I to assess the phytotoxic potential of a pesticide on a minimum number of species. The advantage of using such data is that it provides phytotoxicity information on up to 30 species at no extra cost to the registrant, and the range of plants includes both crop and weed species. However, such data are not generated to the standards of Good Laboratory Practice, which many regulatory authorities now require for laboratory studies. There are also the problems of lack of standard methodology for screening data, and that crop and weed species may be less sensitive to pesticides than non-target species from adjacent habitats, where exposure may be through drift rather than direct overspraying.

It has already been suggested that all pesticides should be tested, and clearly some of these will have no phytotoxic action. Tier I testing for other non-target groups, eg birds, aquatic life, typically uses simple laboratory tests on few species, these being surrogates for the range of non-target species that may be exposed. Although such an approach has been reasonable in determining whether further testing is necessary for a particular group, such as birds or fish, for plants it is sometimes difficult to extrapolate results from one species to others, unless they belong to the same genus (Fletcher, 1991). For this reason there is a need to test a wider range of species for plants at Tier I than is generally necessary for animal groups, and these would need to cover a range of taxonomic groups, ie genera and families. This consideration would entail considerable costs in testing if all pesticides were tested in standard laboratory tests to Good Laboratory Practice. An alternative would be to allow submission of single dose plant screening data (at or above the maximum application rate) on a wide range of species, eg 10-30, to determine whether the pesticide exerts any phytotoxicity. Tests on algae would use standard laboratory test methods (OECD, 1984; ASTM, 1991), but further discussion is needed on the number of species to be tested. Swanson *et al* (1991) conclude that a species battery approach is preferable to the selection of single indicator species.

In all cases if a pesticide is shown to be phytotoxic, ie at least 25% effect in any vascular plant species tested or 50% effect in algae or *Lemna* spp, then Tier II testing would be required. In Canada registrants will be allowed to submit screening data where a range of doses have been used. Allowing submission of screening data at Tier II may cause some difficulties in Europe, as there is a requirement for all ecotoxicity laboratory studies started after 1 January 1992 to be generated according to Good Laboratory Practice (EC directive 87/18/EEC). The US EPA require testing of at least those species affected at Tier I, using a range of doses. At Tier II the UK may require further laboratory data, mesocosm or field data depending on what information is already available on the herbicide, these requirements are discussed case-by-case.

The aim at Tier II for the US EPA is to determine effect concentrations for species, genera or families that may be sensitive to pesticides shown to be phytotoxic. Whereas for Canada the aim is to quantify the magnitude of toxicity to different groups of plant species. This requires generation of dose-response data for a number of species. Endpoints that are currently used are germination, seedling emergence (USA only) and effects on growth/vigour. There was some discussion at the session in Cincinnati as to whether seed germination was necessary as an endpoint in addition to seedling emergence. The present range of species used in testing would need to be extended to meet the aims of testing and to be representative of non-target plants likely to be affected. Only the Canadian guidelines include testing of rooted aquatic macrophytes, at Tier III, however work is needed to develop suitable protocols. There is further discussion of choice of species for testing in this volume (Cole *et al*, 1993). Also a case has been made to increase the number of endpoints considered to include reproductive effects, although a method is not yet available (Fletcher *et al*, 1993). It would seem reasonable to include this endpoint in plant testing if a suitable test method is developed.

Exposure Estimation

An accurate prediction of the exposure of non-target species to a pesticide in the field can be difficult to estimate. Often regulatory authorities differ in the figures they use for exposure estimates, thus a concern may be triggered by one authority but not by another.

For exposure in surface waters, a range of depths are used in estimates from 100 cm by the UK, 30-100 cm in the rest of Europe, to 15 cm in the USA and Canada. The latter is probably the worst case in terms of surface waters that would usually support vascular plants. Harmonisation could be achieved by using 15-30 cm in exposure estimates, discounting the extreme of 100 cm.

For soil depth 5 cm is used in the UK and the rest of Europe for estimates of exposure to soil organisms. This difference, compared to the 3 cm used by Canada and the US, should not cause major difficulties for harmonisation.

Deposition of direct overspray and spray drift is more problematical as this is the area of greatest difference between regulatory authorities. Harmonisation should be based on good data on deposition obtained in the field from properly conducted trials.

The use of uncertainty factors also differs between authorities, only Canada uses uncertainty factors during risk assessment to trigger the next stage of testing, ie

$$\frac{\text{Toxicity}}{\text{Exposure}} = 10 \text{ or less}$$

In view of the lack of knowledge of the effects of pesticides on non-target plants it would be sensible to use an uncertainty factor at least until further experience has been gained of extrapolation of laboratory data to the field.

Multispecies/Field testing

The US EPA Tier III involves testing a diversity of plant groups, regardless of the concerns raised by Tiers I and II, as opposed to the Canadian and UK guidelines which use expert judgement to focus on a specific concern raised by a given pesticide.

CONCLUSIONS

As a result of the session in Cincinnati and comparison of different regulatory schemes, some conclusions can be reached about the key points in moving towards harmonised testing. These are that plants play a critical role in terrestrial and aquatic ecosystems and thus should be protected from the adverse effects of pesticides. However, to achieve environmental protection plant toxicity testing should use ecologically relevant species or plant groups. A tier testing approach should be used to avoid generation of data unnecessary to the risk assessment. Terrestrial and aquatic species should be used in testing, with a range of genera used from various groups, taking account of effects on vegetative growth and reproductive processes. Further research is needed to develop appropriate tests and exposure estimates. A need was identified for post-registration monitoring to validate risk assessment and to detect unpredicted effects.

The importance of a joint international effort at harmonisation was emphasised. Regulatory agencies, industry and researchers should collaborate to produce solutions acceptable to all in order to achieve cost-effective non-target plant protection. This will be of benefit to both regulators and industry, and will also be positively perceived by the public.

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RATIONALE FOR THE CHOICE OF SPECIES IN THE REGULATORY TESTING OF THE EFFECTS OF PESTICIDES ON TERRESTRIAL NON-TARGET PLANTS

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ABSTRACT

Methods are available for the routine glasshouse testing of chemicals on terrestrial non-target vascular plants, but questions still remain over the choice of test species. Considerations for species selection include (a) whether they are representative of the majority of species likely to be exposed to agricultural sprays or are considered to be endangered, and (b) their ability to be grown and assessed reliably under experimental conditions. Analysis of the relationship between the number of endangered species and family size indicated that the most species-abundant families are also representative of threatened species. This has enabled the construction of a prioritised list of plant families from which test species can be selected. Further selection of species from this list was based on their performance under glasshouse conditions and resulted in a pool of 14 species from 10 families suitable for regulatory testing.

INTRODUCTION

There is concern over the hazard that pesticides, herbicides in particular, may cause to wild plants, particularly to those that are either endangered or threatened or in areas of conservation interest (Marrs *et al*, 1989). The use of conservation headlands around field edges (Marshall, 1987; Marshall & Smith, 1987) and "Set-aside" in Europe (Froud-Williams,1988) has also increased interest in herbicide effects on wild plants both within and directly adjacent to crop target areas.

Standard testing methods have been developed for terrestrial plants for the purposes of pesticide registration (Holst & Ellwanger, 1982; OECD, 1984; Brown & Farmer, 1991). A variety of crop species have been tested routinely due in part to concerns over the potential for effects on crops adjacent to the target area. Neither the US EPA, US FDA or the OECD recommended lists for testing contain non-crop species (Fletcher, 1991). The question remains therefore as to how species for regulatory testing should be selected, bearing in mind that they should be representative of both common and threatened species likely to be found in agricultural situations.

Species selection is a three stage process; (a) determination of the taxonomic category from which plants can be chosen, (b) testing of a range of representative species for glasshouse suitability and (c) evaluation of the performance of the selected species for variability and herbicide sensitivity. The first two stages are discussed in this paper.

SPECIES SELECTION PROCESS

Plant family selection

From data supplied by the World Conservation Monitoring Centre, the top ten vascular plant families from North America (from 224 families) and Europe (from 175 families) were first ranked separately according to the total number of species and the number of threatened species each contained. For this paper, the term threatened is taken to include all species coded with the IUCN (International Union for Conservation of Nature and Natural Resources) Red Data Book categories "Extinct", "Endangered", "Vulnerable", "Rare" and "Indeterminate" at the world level.A final ranking for each geographical area was then constructed by summation of the two initial rankings; the ranking for each area is presented with relevant values in Table 1.

With selection from large species-rich families it could be assumed that test representation would be biased in favour of more common species and that rare species would be under-represented. Correlations were therefore carried out on the North American and European data to investigate the relationships between family size and the number (Figure 1) and proportion of threatened species per family.

TABLE 1. The top 10 highest ranking families in North America (NA)[1] and Europe (Eu)[2]. The italicised values in () fall outside the top 10 for either NA or Eu.

Family	Species		Threatened[3] species		Percent threatened[3]		Final family ranking[3]	
	NA	Eu	NA	Eu	NA	Eu	NA	Eu
Campanulaceae	(*110*)	211	(*13*)	77	(*12*)	36	(*34*)	10
Caryophyllaceae	(*288*)	655	(*42*)	161	(*15*)	25	(*13*)	2
Compositae	2233	1613	326	288	15	18	1	1
Cruciferae	511	680	153	141	30	21	5	3
Cyperaceae	718	(*502*)	65	(*7*)	9	(*1*)	6	(*18*)
Gramineae	1478	819	129	69	9	8	3	6
Labiatae	(*320*)	452	(*62*)	107	(*19*)	24	(*11*)	5
Leguminosae	1224	1330	212	105	17	8	2	4
Liliaceae	391	391	86	73	22	19	7	9
Polygonaceae	413	(*104*)	86	(*9*)	21	(*9*)	8	(*27*)
Rosaceae	579	(*480*)	58	(*28*)	10	(*6*)	9	(*11*)
Scrophulariaceae	632	361	154	120	24	33	4	7
Umbelliferae	319	431	81	83	25	19	10	8

1 Includes Canada, Greenland, Alaska and the remainder of the United States (excluding Hawaii).
2 Includes continental Europe (with western parts of Turkey, Russia and the Ukraine), Scandinavia, Mediterranean islands, the Faeroes, the Azores and Iceland.
3 See text for explanation.

Testing species for glasshouse suitability

The first stage in testing species suitability from commercially availably stock is to determine germinability; this is particularly important for tests with pre-emergence spray treatments where reliable and rapid germination is paramount. Other aspects such as ease of assessment and plant variability can be investigated once growth is established.

Studies were carried out by ZENECA (formerly ICI) Agrochemicals to test the suitability of a selection of species from a variety of families. Initially, 19 species were tested alongside the 10 species in use at the time by the company for regulatory testing in order to compare performance; the full list of species tested is given in Table 2. Together, these represented 12 of the families given in Table 1. This was followed by a repeat test with selected species under slightly modified conditions in an attempt to improve performance. For each species, eight replicates, each of 10 seeds per pot were sown in the two experiments; the soil conditions in Test 1 were 4-6% organic matter with seeds sown to 2cm depth, while in Test 2 soil with 1-2% organic matter was used with seeds sown to 1cm depth. Glasshouse conditions were maintained as close as possible to the natural climate of the test species with a 16hr photoperiod for all. Percent seedling emergence and time taken for full emergence of those that germinated were measured. Supplementary measurements of growth stage and plant height were also recorded with notes on general plant vigour and assessability.

TABLE 2. Germinability of candidate species for non-target plant testing - Tests 1 and 2.

Family	Species	Test 1		Test 2	
		% emerged	Days to all emerged	% emerged	Days to all emerged
Chenopodiaceae	*Beta vulgaris**	96	8	/	/
Compositae	*Bidens pilosa*	14	12	10	13
Compositae	*Lapsana communis*	0	-	/	/
Compositae	*Sonchus oleraceous*	35	9	32	10
Compositae	*Taraxicum officinale*	0	-	/	/
Compositae	*Xanthium strumarium**	51	13	97	12
Convolvulaceae	*Ipomoea hederacea**	100	4	/	/
Cruciferae	*Brassica napus**	92	8	/	/
Cyperaceae	*Cyperus rotundus**	91	12	/	/
Gramineae	*Agrostis tenuis*	62	10	/	/
Gramineae	*Avena fatua**	50	8	35	8
Gramineae	*Dactylis glomerata*	32	13	45	13
Gramineae	*Festuca pratensis*	74	9	64	13
Gramineae	*Phleum pratense*	22	13	74	8
Gramineae	*Triticum aestivum**	99	7	/	/
Gramineae	*Zea mays**	97	5	/	/
Labiatae	*Galeopsis tetrahit*	2	12	0	-
Labiatae	*Lamium purpureum*	1	9	0	-
Leguminosae	*Glycine max**	79	9	/	/
Liliaceae	*Allium cepa*	0	-	88	11
Liliaceae	*Asparagus* sp.	0	-	65	16
Malvaceae	*Abutilon theophrasti**	84	4	/	/
Scrophulariaceae	*Veronica hederifolia*	2	12	/	/
Scrophulariaceae	*Veronica persica*	44	10	19	8
Scrophulariaceae	*Rhinanthus minor*	1	7	0	-
Scrophulariaceae	*Scrophularia nodosa*	0	-	/	/
Umbelliferae	*Aethusa cynapium*	0	-	/	/
Umbelliferae	*Daucus carota*	77	13	54	10
Umbelliferae	*Heracleum sphondylium*	0	-	/	/

* Species routinely used in regulatory testing by ZENECA Agrochemicals.

From the first test, 14 species were considered suitable for the second test, either as a result of satisfactory performance or where poor performance was believed able to be improved through changed conditions. The results for germinability from both tests are presented in Table 2. The descision process for final species selection based on the second test are given in Table 3.

TABLE 3. Decision process for final selection of test species from Test 2.

Family	Species	Growth	Assessability*	Decision†
Compositae	*Bidens pilosa*	Strong	A	Reject
Compositae	*Sonchus oleraceous*	Variable	B	Reject
Compositae	*Xanthium Strumarium*	Vigorous	A	Current test sp.
Gramineae	*Avena fatua*	Vigorous	A	Current test sp.
Gramineae	*Dactylis glomerata*	Mortality high	A	Reject
Gramineae	*Festuca pratensis*	Fine structure	B	Potential test sp.
Gramineae	*Phleum pratense*	Vigorous	A	Potential test sp.
Labiatae	*Lamium purpureum*	None emerged	-	Reject
Labiatae	*Galeopsis tetrahit*	None emerged	-	Reject
Liliaceae	*Allium cepa*	Vigorous	A	Potential test sp.
Liliaceae	*Asparagus* sp.	Fine leaflets	C	Reject
Scrophulariaceae	*Veronica persica*	None emerged	-	Reject
Scrophulariaceae	*Rhinanthus minor*	None emerged	-	Reject
Umbelliferae	*Daucus carota*	Strong	A	Potential test sp.

* A = good, B = fair, C = difficult.
† Germinability also included in the decision (see Table 2).

DISCUSSION

The first step in selecting plants for testing the effects of herbicides on non-target species is deciding the criteria that will give the tester a range of species that can be reasonably considered to be representative of those that are likely to be at risk from agricultural sprays. Ideally, the end result of the selection process should be a small group of species encompassing both crop and non-crop plants in order to standardise the testing procedure as far as possible. The relevance of the family level as a starting point for species selection is one that is open for debate. Although the responses of plants to toxicants are not always predictable within families (Fletcher, 1991), the family is the lowest level of taxonomy that can be taken to be representative of plants in general with the test being impracticably large. Families are also fairly stable divisions as far as taxonomists are concerned, particularly those 200 or so within the Angiospermae that are considered to be the "core" families (Heywood,1979).

Assuming then that the family level is a reasonable initial stage for species selection, the next question is what criteria should be set for deciding which families to chose for species selection? The data on vascular plants assembled for this paper indicate that the most species-abundant families are also representative of threatened species. The relationships of family size to the number of threatened species per family, presented for North America in Figure 1, give significant positive correlations (R^2 = 0.74, n= 224 for North America and R^2 = 0.75, n= 174 for Europe). It is reasonable therefore to consider family size as a suitable pre-requisite for species selection.

The second step in species selection, certainly for testing under glasshouse conditions, involves mainly practical considerations. There are four main criteria for acceptability of species for glasshouse regulatory testing; these are (i) percent emergence - 60% or greater, (ii) time taken to fully emerge - 14 days maximum, (iii) general vigour and (iv) ease of assessment.

Only four of the 19 new species tested in the study reported here were considered as potential candidates for inclusion in the test group; see Table 3. This highlights the difficulty of species selection for the constrictions of routine testing. Most (12) were primarily rejected on the grounds of poor germinability, though general vigour and ease of assessment were contributory factors in some cases. Germination, however, need not be a critical factor on the basis of a single

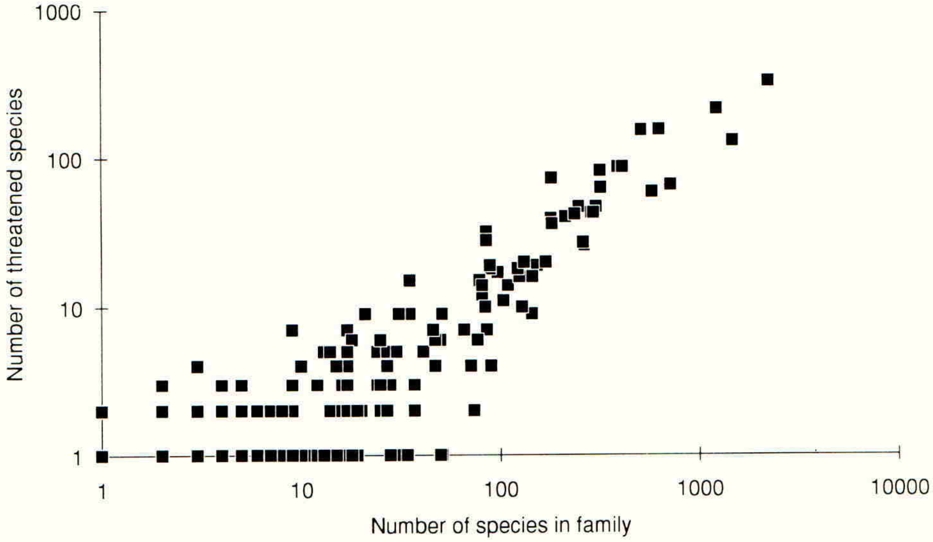

FIGURE 1. Relationship of family size to number of threatened species in North America.

test; as with *Asparagus* sp. in this study, germination can be improved in some cases by altering the planting conditions. Assessability is a very subjective evaluation based on experience of working with different plant structures and whether consistent measurements of damage can be made; a number of points were considered, e.g. ease of management (not spreading or climbing, excessive tillering), presence of natural epidermal blemishes and very fine structure making the detection of low damage difficult.

TABLE 4. Plant test species pool used by ZENECA Agrochemicals for regulatory testing.

Family	Species	Common name	Crop/non-crop
Chenopodiaceae	*Beta vulgaris*	Sugarbeet	Crop
Compositae	*Xanthium strumarium*	Cocklebur	Non-crop
Convolvulaceae	*Ipomoea hederacea*	Morning glory	Non-crop
Cruciferae	*Brassica napus*	Oilseed rape	Crop
Cyperaceae	*Cyperus rotundus*	Purple nutsedge	Non-crop
Gramineae	*Avena fatua*	Wild oat	Non-crop
Gramineae	*Festuca pratensis*	Meadow fescue	Non-crop
Gramineae	*Phleum pratense*	Timothy	Non-crop
Gramineae	*Triticum aestivum*	Wheat	Crop
Gramineae	*Zea mays*	Maize	Crop
Leguminosae	*Glycine max*	Soybean	Crop
Liliaceae	*Allium cepa*	Onion	Crop
Malvaceae	*Abutilon theophrasti*	Velvet-leaf	Non-crop
Umbelliferae	*Daucus carota*	Carrot	Crop

The pool of test plants from which ZENECA Agrochemicals selects for Tier 1 and 2 regulatory testing now consists of 14 species from 10 families, as listed in Table 4. These can be considered to be representative of a wide range of potentially non-target species, both crop and non-crop. The list is still, however, somewhat over-represented by crop species (50%) and the Gramineae (36%).

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ENVIRONMENTAL INTERACTIONS OF PESTICIDES: SYNERGISM OF PERMETHRIN BY SIMAZINE AGAINST THE HOUSEFLY

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ABSTRACT

In preliminarily studies of the interactions between herbicides and insecticides on insects, the adult housefly (*Musca domestica* L.) was chosen as a representative insect. Simazine was administered to a susceptible (Cooper) strain of the housefly topically and by injection, alone or in mixtures with permethrin. The effect on permethrin toxicity of piperonyl butoxide (PB) was also investigated and compared with simazine. Unsynergised permethrin and simazine gave topical acute LD50 values of 10 ng and 6μg/fly after 24 hours respectively. When applied in a mixture simazine synergised permethrin at ratios 50:1 and greater. PB also showed synergism but at lower ratios to permethrin than that of simazine. The co-toxicity coefficients were estimated and isoboles were plotted to analyse and compare the interaction in both cases.

INTRODUCTION

The effects of pesticides or other synthetic chemicals and natural toxins on biological systems have usually been investigated primarily with a single chemical. For pesticide combinations, the study of synergy began by the assessment of acute lethal effects. Independent joint action refers to poisons acting quite independently on different physiological mechanisms. Synergism is the special case of joint action where one of the ingredients is non-toxic at the doses used, but has the effect of increasing potency of the other component of the mixture.

A study of insecticide/herbicide synergism is also warranted from an environmental perspective. It is already known that such joint action does occur in some non-target species (Fisher, 1992). It is also clear that many pesticides are present in soil and ground and surface waters due either to direct application or to dispersion from the orginal area of application. Some contaminants found in surface and soil waters include parathion, malathion, carbaryl, carbofuran, atsazine, simazine and 2,4-D (Crathorne *et al.*,1984). It is possible that a reduction in populations of beneficial organisms or shift in species diversity are exacerbated as a result of insecticides/herbicides joint action (Fisher, 1992). If the impact of pesticides in the environment is to be evaluated accurately and if it is desirable to be able to predict the hazard associated with pesticide application, it is critical to have understanding of how these chemicals interact with one another on exposed organisms. To assist with such understanding the present investigation was done to assess the interaction between simazine and permethrin on the housefly (*Musca domestica* L.).

MATERIALS AND METHODS

Chemicals

Permethrin

Permethrin (ICI Agrochemicals) [3-phenoxybenzyl (1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, 94.1% technical, cis trans ratio 40:60 was used. Methanol was used as solvent. Bioassays were carried out by topical application of a 1μl drop of a solution of different concentrations to the thoracic notum of 3-days old adult male and female flies with a microapplicator. The flies were anaesthetized with carbon dioxide before dosing. Anaesthetized flies are held with padded entomological forceps for treatment. Five replications were made including a control batch of flies treated with methanol only.

Simazine

Simazine (technical grade) was from Griffin, Valdosta,GA [2-chloro-4, 6-bis (ethylamino)- s-triazine]. For topical application simazine was first dissolved in dimethylsulphoxide (DMSO), then diluted in methanol. The method of topical application used was as that for permethrin. For injection a suspension was made by sonicating simazine in distilled water for 5 minutes and injected into 3-days old anaesthetized flies. For this the anaesthetized fly was held with a fine forceps and injected with a syringe (needle size G30 x 1 cm) fitted in a microapplicator. The injection (1μl) was given at the lateral side of the abdomen carefully avoiding any puncture of the viscera. All experiments were replicated five times including control.

Combined doses

Four dosages of permethrin (2.9, 5.9, 8.9 and 11.9μg/fly) were applied topically on 3 day old houseflies in mixtures prepared in mass ratio of 1:1, 1:2, 1:5, 1:10, 1:20, 1:50, 1:100, 1:200, 1:500, 1:1000 with simazine and the LD50 for each ratio was calculated.

To compare the synergism of piperonyl butoxide (PB), a known synergist of permethrin, the same concentrations of permethrin were used and bioassays were done in the same manner as simazine.

Insect handling procedures

Cooper, a pyrethroid susceptible strain of housefly (Chapman and Morgan, 1992) was used. The stock cultures were maintained at 25°C in a mesh cage (50 x 30 x 30 cm). The adults were provided with milk-sugar solution as food. Adult female flies laid eggs in a food cup (250 ml) having larval food(yeast, milk powder and bran at ratio of 1:2:10 mashed in 500ml of distilled water). The eggs were laid on the food and the cups were removed from the oviposition cage and placed in another cage at the same temperature. Three day old adults flies were used in the experiments. Before each bioassay the flies were starved for 6 h.

Analysis of data

Mortality of the treated flies was recorded after 24 hours. Probit analysis was done according to Busvine (1971). Co-toxicity coefficients were calculated as for Sun & Johnson (1960). Isoboles were drawn using the Fig-P (Biosoft) package by freehand curve fitting.

RESULTS

Single treatments

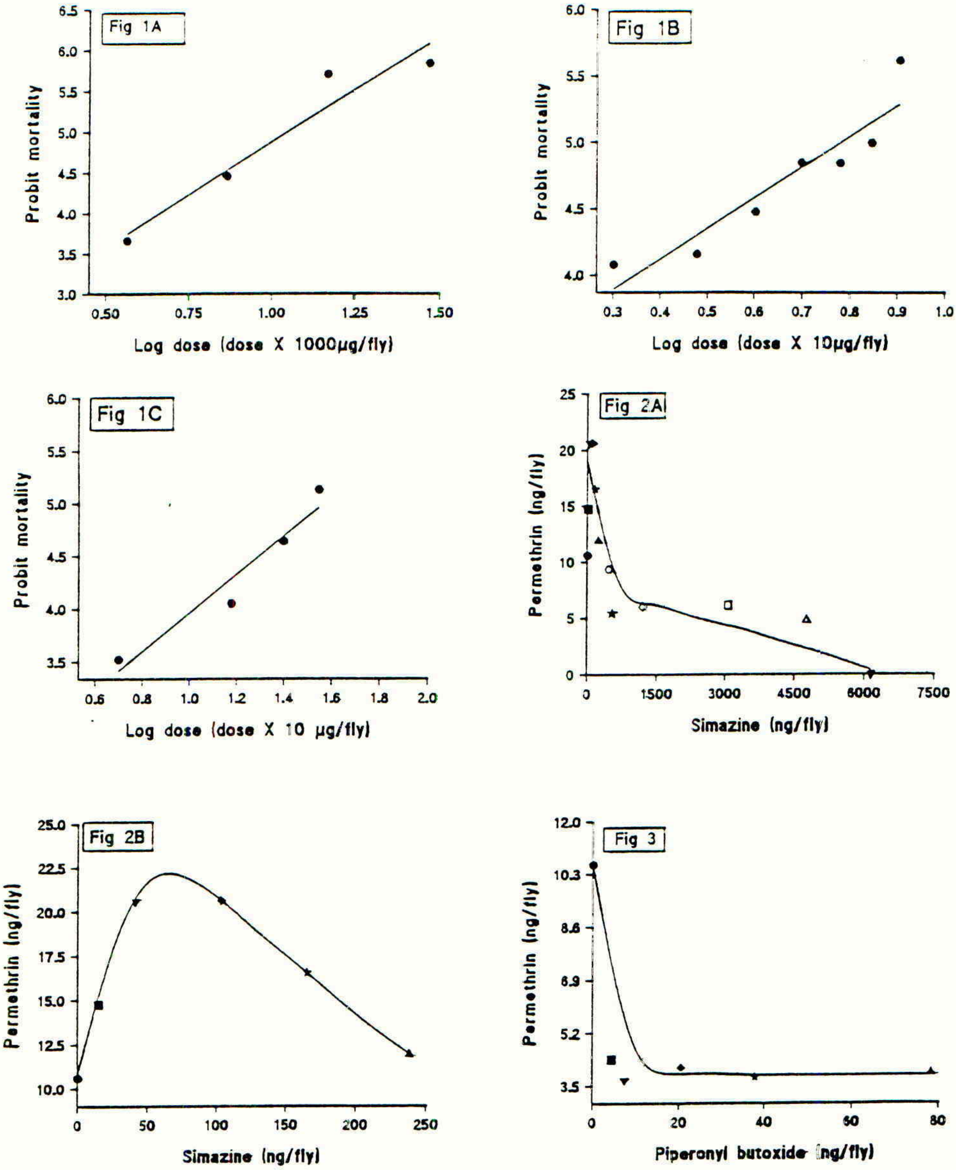
Initial bioassays were carried out to established the effect of single treatments. The topical LD50 for adult housefly was 10.6 ng/fly (1.15 ng/mg) for permethrin treatment, whereas it was 6147 and 3699 ng/fly (575 and 321 ng/mg respectively) for simazine treatment topically and by injection respectively (Table 1). The regression lines are presented in Figure 1. In the present investigation the injection was done using a suspension of simazine in distilled water as the use of methanol or DMSO substantially increased the mortality of the flies (in the control). It appeared that simazine was able to penetrate the cuticle readily as the LD50 when applied topically was only 1.7 times that when injected.

TABLE 1. LD50, confidence limits and regression equations.

Chemical	LD50 (ng/fly)	Confidence limit (ng)	Regression equation	X^2 (df)
Permethrin	10.6	8 - 13	Y = 2.307 + 2.617X	2.89 (2)
Simazine (topical)	6147	4470 - 8453	Y = 3.185 + 2.3X	1.65 (5)
Simazine (injected)	3669	1860 - 7235	Y = 2.018 + 1.905X	0.57 (2)

Combined treatments

The mixtures of permethrin and simazine were used in different ratios keeping permethrin constant at 7.3 ng/μl. The results (Table 2) indicate that simazine has some synergistic effect with permethrin on the housefly. The cotoxicity coefficients shows that when applied in a mixture simazine synergised permethrin at ratios 50:1 and greater. Below this the LD50 for permethrin with simazine in mixture showed antagonism. The LD50's of both chemicals are plotted in Figure 2 and isoboles suggests that at lower ratios (Figure 2B) there is some antagonism but as the amount of simazine in mixture increased the synergism occurs (Figure 2A).



●–LD$_{50}$(P), ■–1:1, ▼–1:2, ◆–1:5, ★–1:10, ▲–1:20, ○–1:50, ★–1:100, ◇–1:200, □–1:500, △–1:1000, ▽–LD$_{50}$(S)

FIGURE 1. Regression lines of probit mortality on log dose of permethrin topically applied (A), simazine topically applied (B) and simazine by injection (C).

FIGURE 2. Isobole of simazine and permethrin; (A) high ratios 1:50-1:1000 and (B) low ratios 1:1 to 1:50.

FIGURE 3. Isobole of LD50 of piperonyl butoxide and permethrin.

TABLE 2. LD50 and co-toxicity coefficients of mixtures of permethrin (P) and simazine (S) or piperonyl butoxide (PB) at different ratios applied topically to the housefly

Chemicals	Ratio	LD50(ng/fly)			Co-toxicity coefficients
		P+S or PB	P	S or PB	
P : S	1:1	29.52	14.76	14.76	71.81
	1:2	61.79	20.59	41.19	51.48
	1:5	123.77	20.62	103.14	51.40
	1:10	181.92	16.53	165.38	64.12
	1:20	250.04	11.91	238.13	89.00
	1:50	477.05	9.35	467.69	113.36
	1:100	547.57	5.42	542.15	195.57
	1:200	1210.76	6.02	1204.73	176.07
	1:500	3071.83	6.13	3065.69	172.92
	1:1000	4761.80	4.76	4757.04	222.08
P : PB	1:1	8.71	4.35	4.35	243.39
	1:2	11.04	3.68	7.36	288.04
	1:5	24.54	4.09	20.45	259.17
	1:10	41.64	3.78	37.85	280.42
	1:20	82.14	3.91	78.23	271.10

The mixtures of permethrin and PB also showed synergism. In this investigation the same ratios were used as for simazine and permethrin and PB synergised permethrin at all ratios with co-toxicity coefficients very high in each case (Table 2). During the experiment it was found that permethrin to PB ratio above 1:20 gave 100% mortality to the houseflies. The isobole (Figure 3) of the LD50 of permethrin and PB shows high levels of synergism.

DISCUSSION

These results demonstrate clearly that the already high toxicity of permethrin to the housefly can be increased significantly when combined with simazine. All *et al.* (1977) reported synergism of the synthetic pyrethroids, permethrin and fenvalerate with methyl parathion and methomyl. Lichtenstein *et al.* (1973) found that the herbicides atrazine, simazine and monuron increased the toxicity of a range of insecticides to the housefly and among them atrazine was most effective, increasing significantly (P = 0.1% level) the toxicity of parathion. The synergistic effect of atrazine with parathion in water or in water with soil were measured with mosquito larvae (*Aedes aegypti* L.) by Liang and Lichtenstein (1974) who observed that soil reduced the effectiveness of the insecticidal effect. Atrazine, which is not toxic by itself, increased the toxicity of insecticides significantly. Lichtenstein *et al.* (1979) conducted further studies on the housefly to test the effects of atrazine on the toxicity of organophosphorus insecticides and found similar results to those observed with carbofuran and atrazine. Degradation in insects of carbofuran was inhibited in the presence of atrazine as observed by Chio and Sanborn (1977), who contended that compounds capable of blocking the microsomal electron flow can potentiate the toxicity of certain insecticides. Similar results were also observed with a fungicide (prochloraz) which increased significantly the toxicity of pyrethroids (Colin and Belzunces, 1992).

The resultant isoboles support the hypothesis that the synergists are similarly at inhibiting the metabolic detoxification (hydroxylation) of the insecticide. The curves for simazine follow the trend that would be expected with an enzyme inhibition system, where with an increased amount of synergist there is an increase in inhibition of the detoxification enzyme system.

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TOXICITY OF SOIL APPLIED HERBICIDES TO BRINE SHRIMP LARVAE (*ARTEMIA SALINA*) AND SYNERGISM WITH OTHER PESTICIDES

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ABSTRACT

The potential effects of selected soil applied herbicides, and their interaction with other pesticides were evaluated using the marine crustacean, *Artemia salina*, the brine shrimp.

Newly hatched *A. salina* larvae were exposed to the herbicides and their mixtures with other pesticides and the acute toxicity determined at 24 hours. The toxicities of the herbicides (LD50; simazine: 23, atrazine: 42, bromacil: 71mg/l) were similar to that of the organophosphate, malathion (37mg/l) but lower than that for the carbamate, carbofuran (1.1mg/l).

The interaction between the herbicides and the two contrasting insecticides was analyzed by co-toxicity coefficients and through plotting isoboles of the LD50 values. These results clearly showed high levels of synergism, with the co-toxicity coefficient increasing with the ratio of herbicide to insecticide. At high herbicide to insecticide mass ratios (1000:1 for carbofuran and 100:1 for malathion) the co-toxicity coefficients were 115000 to 128000 and 11900 to 13800 respectively. The synergism was most marked at low concentrations of the insecticides, especially with carbofuran. The lethal action of the insecticides was also synergized by the natural product, caffeine.

INTRODUCTION

The responses of biological organisms, at the individual and population levels, to pesticide residues, may be influenced by the presence of other biologically active substances. These could include other pesticides, their metabolites, chemical pollutants including gases and natural substances. The presence of mixtures of pesticides and metabolites in certain parts of the environment, especially soils and surface and ground waters, is being continually documented (Bushway *et al*, 1992). The reaction of organisms to these mixtures may not be predicted from a knowledge of the toxicology of the components. Although this may not be true of many combinations evidence is emerging of possibly detrimental interactions with herbicides in the case of invertebrate animals (Douglas *et al*, 1993).

The herbicides atrazine, simazine, monuron and 2,4-D were found to increase the toxicity of a range of insecticides to the housefly, *Musca domestica*, mosquito larvae, *Aedes aegypti* and *Drosophila* (Lichtenstein *et al*, 1973). Later studies (Lichtenstein *et al*, 1979) showed that atrazine reduced the uptake and metabolism of carbofuran in houseflies. These effects were also observed in the house cricket *Acheta domesticus* (Chio and Sanborn, 1977).

To evaluate the interaction between nitrogen heterocyclic herbicides and insecticides a marine crustacean, the brine shrimp, *Artemia salina*, was chosen, because it can represent an important high risk group (aquatic crustacean). In addition it is used considerably for evaluating the toxicology of natural products (Alkofahi *et al*, 1989), the eggs are readily available and the bioassays with it can be done conveniently at a microscale. The purpose of this investigation was to characterize the acute lethal response of the susceptible larval stage to mixtures of well established soil applied herbicides (and the heterocyclic plant natural product, caffeine) and two dissimilar insecticides, carbofuran (carbamate) and malathion (organophosphate).

MATERIALS AND METHODS

Materials used

The pesticides used were atrazine (technical grade, Griffin, Valdosta, GA, USA), simazine (technical grade, Griffin), bromacil (technical grade, Griffin), carbofuran (technical grade, Bayer AG, Leverkusen, FRG) and malathion (technical grade, Farm Protection, Oakham, UK). The natural plant product, caffeine (anhydrous, Sigma) was included.

Brine shrimp larvae

Brine shrimps were reared from eggs in aqueous sodium chloride (15g/l) in a small glass tank, at 30^0, fitted with a mesh divider. The tank was partially masked with light-proof material such that as the eggs (placed on the dark side of the partition) hatched the larvae moved through the partition where they could be collected free of eggs and eggshells. Unfed larvae up to 24 h old were used.

Bioassay methods

The bioassays were done in microtitre plates with wells (96 per plate) having a capacity of 300μl. Using a dissecting microscope, shrimp larvae were collected from the rearing tank with a piston micropipette (taking 75μl brine and 5 shrimps) and transferred to each well of the microtitre plate. Subsequently, 75 μl of the test solution (in double distilled water) was added to each well as appropriate. The treatments were individual chemicals, or their mixtures at constant mass ratios, at a range of concentrations (6-8) to give mortality responses between 0 and 100%. The solutions of the individual chemicals were prepared by weighing out the technical grade and preparing stock solutions using methanol (for carbofuran, atrazine and simazine), acetone (for malathion and bromacil) and water (for caffeine). The concentrations required for the bioassay were obtained from the stock solutions by serial dilution with distilled water. Controls of the appropriately diluted solvent showed no observable effect of the trace of solvent. A randomised block layout was used with 8 replicates (four replicates in the cases of the mixtures). Following the application of the pesticide solutions the plate was covered with clingfilm and placed in a dark incubator at 30^0.

Treatments and analysis

The treatments were each of the chemicals alone and mixtures of carbofuran or malathion with the three herbicides and caffeine. For all the ratios of each insecticide with the other compounds (see Table 2) a range of concentrations was chosen to give 24 h lethal action between 0 and 100%, corrected for control mortalities, as needed. The log concentration against probit percent mortality was plotted to give the LC50 values. These values were used to calculate the cotoxicity coefficient (Sun and Johnson. 1960) and to plot isoboles of the LC50 for each of the mixtures.

RESULTS

The 24 hour acute toxicity for each of the single compounds for brine shrimp larvae is given in Table 1. The survival of the larvae (untreated) was optimized by using only newly emerged larvae, within 14 hours of hatching. The results of the mixtures of the two insecticides with the herbicides (and caffeine) are given in the form of co-toxicity coefficients in Table 2.

TABLE 1. Acute toxicity (LC50) of individual compounds to brine shrimp larvae

Compound	LC50 mg/l
carbofuran	1.11
simazine	23.12
malathion	37.03
atrazine	41.78
caffeine	52.73
bromacil	71.16

TABLE 2. The co-toxicity coefficients for mixtures of two insecticides at various ratios with three herbicides and caffeine.

Ratio of insecticide to synergist	Synergist: simazine	caffeine	bromacil	atrazine
carbofuran:				
1:01	279	282	308	278
1:05	1107	895		1314
1:10	2416	2374	2013	2153
1:20	4067			6252
1:50	8258	11804	14223	17468
1:100	16924	29880		22823
1:200	29756	35154	39805	44345
1:500	66801	59456		67810
1:1000	129252	102749	121538	114523
malathion:				
100:01	105	114	119	114
10:01	243	233	156	148
5:01	311	511	319	240
1:01	1611	595	401	1126
1:10	4087	1942	1587	2142
1:50	9948	7684	10701	11014
1:100	13139	10528	13788	11893

The LD50 values were obtained for each binary combination at each ratio in Table 2 from each log dose-probit regression (data not given). For each mixture the amounts of the components to give the 50% lethal response were plotted in the form of an isobole (Hewlett, 1960) as shown in Figure 1 for interactions with carbofuran, and in Figure 2 for malathion. The points for the 50% lethal response of the individual pesticides (as in Table 1) provide the extremes of a free hand fitted line (FigP software; Biosoft) with the plots for additive action represented by the dashed lines. In all cases the points fall beneath this dashed line and represent synergistic (potentiation) interactions between the pesticides, and also for caffeine. As shown by the co-toxicity coefficients, at high ratios of the herbicides there were high levels of synergism of the brine shrimp mortality. Values over 100 indicate synergism or potentiation, which term may be used in the cases where both components of the mixture caused appreciable toxicity.

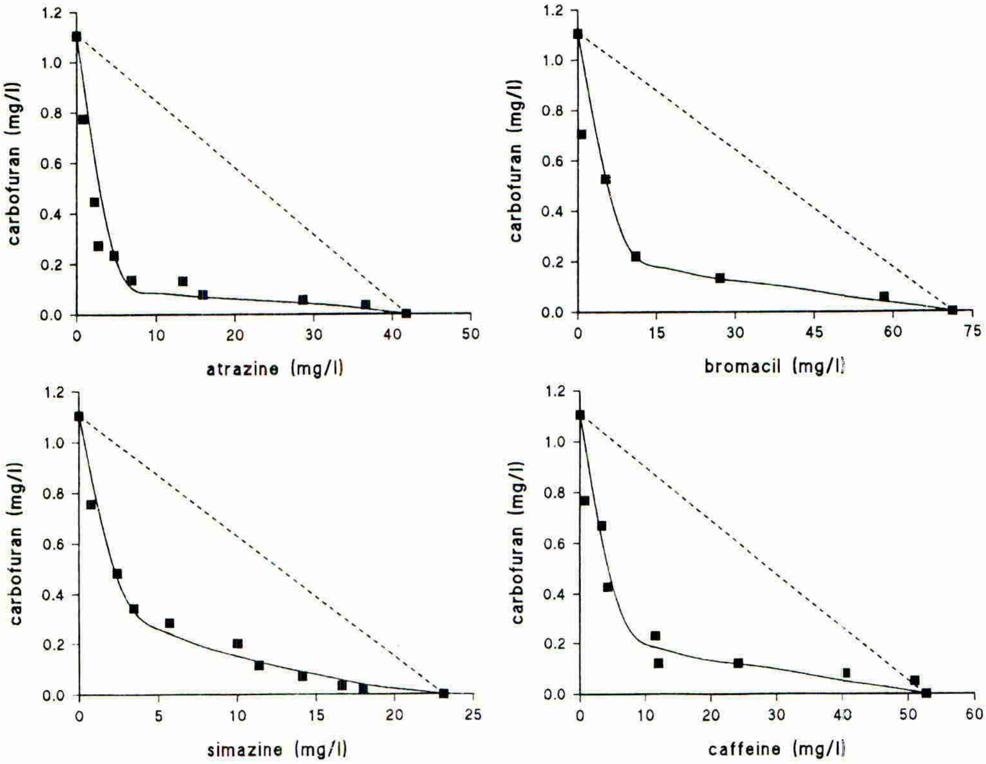

Figure 1. Isoboles for the 50% lethal responses to mixtures (carbofuran) by brine shrimp larvae. The dashed lines indicate values for additive action.

DISCUSSION

Toxicities of individual compounds

The toxicities of the triazine compounds are in the same order as observed by

Marchini *et al*, 1987 who reported values of 1.1mg/l (simazine) and 6.9mg/l (atrazine) using the freshwater crustacean, *Daphnia magna*. Other estimates in this paper indicated values of the LC*50* above the solubilities of the compounds. Using the method given in our work for preparing the treatments (increasing dilution of methanolic solutions of simazine especially) higher concentrations can be obtained without harmful effects to the brine shrimp under the experimental conditions. In the case of simazine this may have been in the form of a suspension but this did not apparently affect the dynamics of uptake. The results showed the high toxicity of simazine (compared to both atrazine and malathion) to this species. The toxicity of caffeine to arthropods is established (Nathanson, 1984) and the results of this study show the moderate level of toxic activity of this trimethylxanthine.

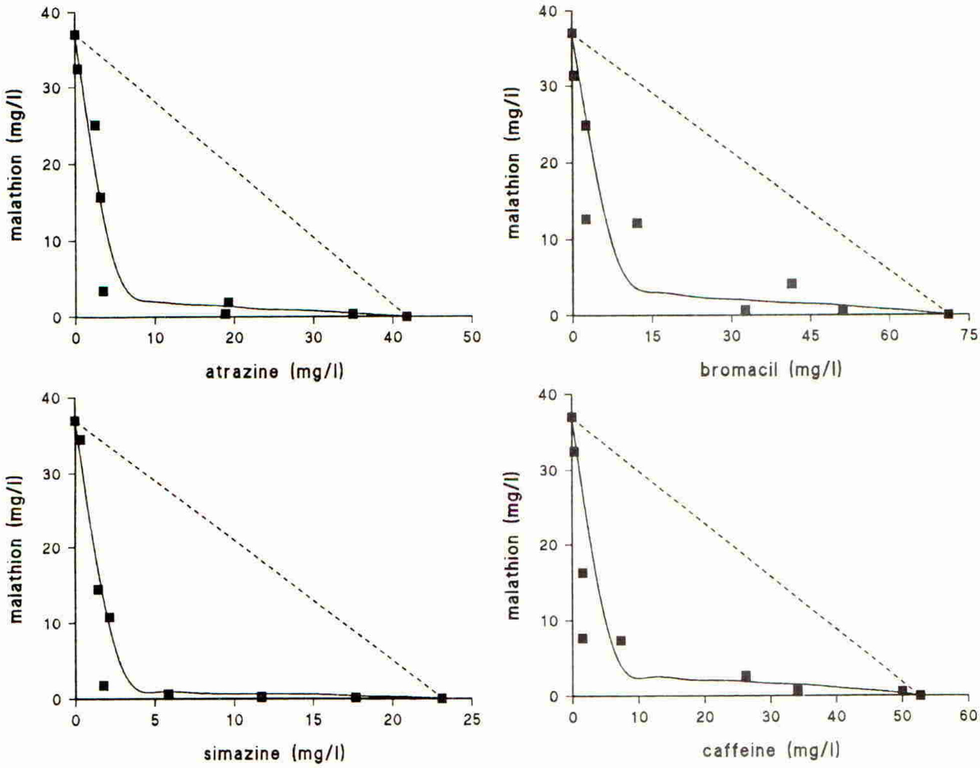

Figure 2. Isoboles for 50% lethal responses to mixtures (malathion) by brine shrimp larvae, see Figure 1 for explanation.

Pesticide interactions and synergism

The results of the bioassays conducted with mixtures of carbofuran and malathion with the three herbicides and caffeine showed greater lethal effect on the brine shrimp larvae than expected from the known individual toxicities. For every binary ratio studied there was synergism (Morse, 1978) of lethality with the isoboles (Figs 1 and 2) and the nature of the interaction conformed with that where the synergist interferes with the dynamics of the main pesticide. However, in some combinations, e.g. malathion and simazine, the herbicide is more toxic to brine shrimp than the insecticide. The mode of action of the simazine is unknown although the structural requirements for triazine toxicity were studied by Marchini *et al*, (1987). The greatest synergistic effects were seen with carbofuran, which has the intrinsic highest activity possibly due to its systemic and mobility properties, particularly in aquatic systems (Trotter *et al*, 1991).

Clearly the degree of potentiation displayed by the three herbicides and caffeine is strong, as defined by Sun and Johnson, 1960 (values significantly above 100). The inclusion of caffeine in this study was to extend the structural requirements for this type of synergism with arthropod lethality, and this has been demonstrated. These requirements may also extend to other natural products, especially certain alkaloids, with some implications for insecticide use on crops. However, there may be some significance on the effects of pesticide mixtures (and metabolites and natural toxins) on exposed non-target organisms. Although ambient concentrations that may give rise to lethal effects may be rare, the responses of brine shrimps and possibly other organisms at sub-lethal concentrations (such as behaviour and reproduction) may also show analogous synergism with such mixtures.

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AERIAL SPRAYING OF ASULAM AND ITS EFFECTS ON BIOASSAY PLANTS IN A DERBYSHIRE CLOUGH

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ABSTRACT

Aerial spraying of asulam is used widely in upland areas to control bracken (*Pteridium aquilinum*) as a precursor to moorland restoration. However, other fern species are sensitive to asulam and may be affected by this policy. These sensitive ferns are often found in small valleys or cloughs which dissect the moorland. In this study the deposition of asulam and its subsequent effects on two species of test plant were assessed in a Derbyshire clough during a bracken control/moorland restoration programme. Herbicide deposition varied markedly at different positions in the clough, but significant damage to the test plants was found throughout. The methods described here appear suitable for helping to design improved spraying strategies in these sensitive areas as well as for policing aerial spraying.

INTRODUCTION

During the last few years there has been considerable disagreement over the policy of using aerial applications of asulam for the control of bracken (*Pteridium aquilinum* (L.) Kuhn) in upland areas. One of the main concerns as far as conservation is concerned is that other ferns, which are often relatively rare, may be affected by asulam use. As most ferns are generally susceptible to asulam (Marrs & Griffiths, 1986; Horrill, *et al*, 1978), it is likely that these ferns will be seriously damaged or killed, if they are present in sprayed areas, or if they are immediately downwind of sprayed areas. There have, however, been few attempts to assess the impacts of asulam use on these ferns under field application conditions.

Within the North Peak Environmentally Sensitive Area (ESA) in Derbyshire, the problems for fern conservation are considerable. The rarer ferns are often found in cloughs, small dissected valleys that cut down from the moorland fells over the escarpments into the lower valleys. However, dense bracken often covers much of the surrounding moorland and extends down the clough sides replacing the moorland vegetation. Seven fern species besides bracken have been found in these cloughs including; Lady Fern (Athyrium filix-femina (L.) Roth.), Hard Fern (Blechnum spicant (L. Roth.), Scaly Male-fern (Dryopteris affinis (Lowe) Fraser-Jenkins), Broad Buckler-Fern (D. dilatata (Hoffman) Gray), Oak-fern (Gymnocarpium dryopteris (L.) Newman), Lemon Scented-Fern (Oreopteris limbosperma (All.) Holub), and Beech-fern (Phegopteris connectilis (Michx) Watt). Preliminary observations suggest some of these rarer ferns may be badly damaged or killed in the year after asulam use.

There is, therefore, a dilemma between the conservation management required to maintain and enhance moorland communities and the conservation requirements of these rare ferns. As a balance has to be struck between these two opposing objectives, there is a need for detailed knowledge on the effects of aerial application of asulam on sensitive species in the types of habitat where these ferns are found. It is possible, for example, that individuals found under dense bracken, or protected by steep slopes or ledges, might not be affected as much as plants found in the open. Moreover, if sensitive areas are to be protected in the future there is the need to develop effective methods of checking for damage arising from aerial spraying campaigns on adjacent land. Such methods could help to (1) provide information to improve spraying methodologies, and (2) to police aerial spray use to ensure that FEPA regulations (Food Environmental Protection Act (FEPA, 1989a,b) are being met.

This paper attempts to address both of these issues by assessing the deposition of asulam and its effectiveness on bioassay plants in a range of different positions in one of the cloughs sprayed by helicopter with asulam in 1990 within the North Peak ESA. Bioassay plants were used in preference to observations on native populations because symptoms of herbicide damage on wild species can often be confounded with damage from other sources, especially if the herbicide damage resulted from sub-lethal doses. Use of standardized material with appropriate untreated controls ensures that effects of other environmental factors are minimized and that any symptoms found can be directly attributed to the herbicide.

METHODS

Plant propagation

Rumex acetosa L. (Common Sorrel) was the main test plant used as it had already been shown to be susceptible to asulam drift (Marrs *et al.*, 1990; Marrs, *et al.*, 1992). After 2 - 3 weeks the leaves of affected plants show severe chlorosis, followed in some instances by necrosis and death. Seeds of *R.acetosa* were sown in January 1990 and then potted individually into 7 cm x 7 cm x 8 cm pots containing SAI GP compost. Developing flowering stems were cut to prevent premature dormancy, and at the time of spraying the plants had between 10 - 40 mature leaves. In addition to the *R.acetosa* plants, a smaller number of the non-native fern *Adiantum pubescens* Schkuhr. was also used. The ferns were obtained from a horticultural supplier in June 1990 and repotted immediately as described above for *R.acetosa*.

Seed trays containing six pots of either *R.acetosa* or *A.pubescens* were used for bioassays in the field.

Experimental layout

The experimental site was at Stable Clough (Grid reference SK 100994) near Glossop, Derbyshire. The site was a steep valley with dense bracken covering the moorland top and extending part way down the clough sides. On the steep sides the bracken gave way to open grassland and rocky outcrops and ledges, and in the bottom there was both *Calluna vulgaris* (L.) Hull heathland and open stony ground. Eight positions, chosen to reflect different types of habitat where the rarer ferns could persist, were established on a transect across the clough: (1) Clough Top - outside bracken canopy, (2) Clough Top - under bracken canopy, (3) Clough Side -open, (4) Clough Bottom - in *C.vulgaris* vegetation, (5) Clough Bottom - in open ground, (6) Clough Side - open stony ground, (7) Clough Top - under bracken canopy, and (8) Clough Top - outside bracken canopy.

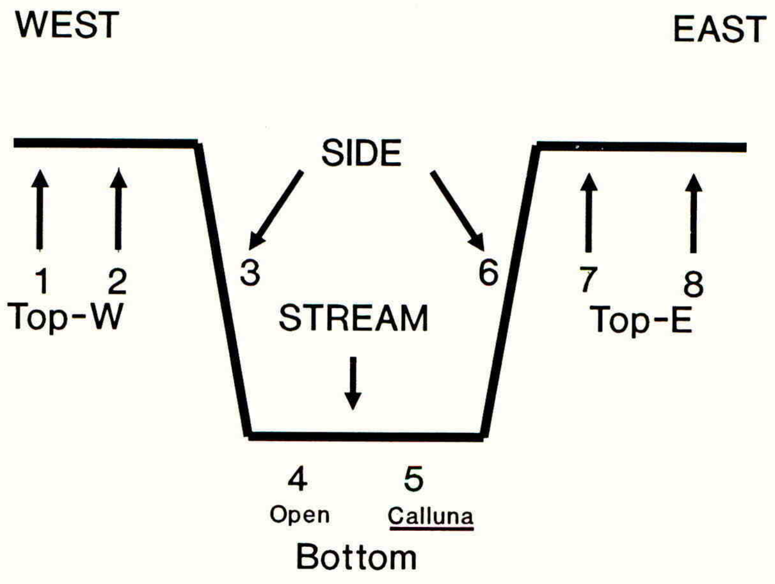

FIGURE 1. Plan of the transect across Stable Clough with eight sampling points: circles = no vegetation cover, squares = under dense bracken. The helicopter applied the asulam at right angles to the transect line, i.e. in a North-South direction.

Five trays of *R.acetosa* and three trays of *A.pubescens*, each with six individuals, were placed around each transect point immediately before spraying. Twelve trays of *R.acetosa* and three trays of *A.pubescens* were kept as untreated 'controls'. Four water-sensitive papers (76 cm x 52 cm) were also placed horizontally at each position (combining positions 4 and 5) to provide a crude assessment of spray drift deposition (Sinha *et al.*, 1990).

The spraying was done by a commercial operator as part of a wider bracken control/moorland restoration programme within the North Peak ESA. Asulam was applied by a Bell 47G3B1 helicopter at a rate of 4.4 kg AI/ha (11 l/ha Asulox) in 44 l/ha spray containing a 0.1% non-ionic wetter (Agral). A 12 m boom was used fitted with 72 Raindrop nozzles and the tank pressure was 2 bar. Flying height was between 5-10 m. The entire clough and its surrounding bracken was sprayed in a series of uphill and downhill swaths across the transect across the clough (Figure 1). Wind speed and direction were monitored continuously during the spraying period using a Vector Instruments R500 recording anemometer. The wind direction was easterly with some gusts from the north-east. Wind speed varied between 2 - 7 m/s at a height of 2 m. There was no rain during the spraying period.

After exposure, the plants were left for 2 h to dry. The *R.acetosa* plants were then transferred to an experimental garden, and the *A.pubescens* were maintained in an unheated glasshouse. After three weeks each *R.acetosa* plant was assessed visually for damage by counting (1) the total number of leaves, and (2) the number of these leaves showing chlorotic or necrotic symptoms. The mean percentage (± SE) of leaves damaged at each transect point was then calculated. After six weeks the foliage of all *A.pubescens* plants in each replicate seed tray was harvested, oven dried at 80°C and weighed.

RESULTS

In the two open areas (positions 1 and 8) at the top of the clough where asulam deposition should be at the full recommended rate similar amounts of deposition (c. 4% of the water-sensitive paper) were detected (Table 1). On the downwind western half of the clough similar amounts were deposited on the clough side (position 3) and on the clough top under dense bracken (position 2). In other areas (clough bottom (points 4,5), eastern side (position 6) and under bracken on the eastern top (position 7) the deposition was much lower, approximately 25 % of the amount found at full exposure (positions 1 and 8).

Damage to *R.acetosa* was severe (> 50%) in all six situations, which was much greater than the 3.5 % found in the untreated controls (Table 1). Damage was highly correlated with deposition rates on water sensitive papers ($r = 0.95$; $n=7$), described by the following equation:

$$Y_{damage} = 8.45\, X_{drift\ deposition} + 50.24 \quad (F=50.7; \underline{P}<0.001)$$

Biological damage was found, therefore, even when the deposition on water-sensitive papers was below the detection limit (c. 0.1% of the water-sensitive paper).

Damage to the *A.pubescens* was less clear cut, although a significantly lower yield than the untreated controls was found at all transect points (Table 1).

TABLE 1. Deposition of herbicide on water sensitive papers, leaf damage assessment of *R.acetosa* and dry weight of *A.pubescens* at the different positions on the Stable Clough transect; mean values (±SE) are presented.

Position on transect across clough			Deposition on water sensitive papers (%) (n=4)	Damage to *R.acetosa* (% of leaves damaged) (n=5)	*A. pubescens* dry weight (g) n=3)
Top	- outside bracken	W	3.6±0.4	77±16	4.0±0.2
	- outside bracken	E	4.3±1.0	83±3	4.9±0.1
Top	- under bracken	W	4.7±0.5	97±2	4.6±0.2
	- under bracken	E	0.8±0.2	50±14	6.1±0.1
Side	-	W	5.9±1.3	99±6	3.3±0.1
	-	E	0.9±0.1	58±4	4.0±0.3
Bottom					
	- under C.vulgaris			68±5	5.9±0.5
			0.9±0.3		
	- in open			64±5	4.6±0.4
Untreated 'controls'			-	3.5±0.4	7.1±0.4

DISCUSSION

A clear result of this study has been that there were considerable differences in the amounts of herbicide deposited in the different positions within the clough. However, even where the lowest amounts of drift deposition were detected, there was still considerable damage to both species of test plant. Moreover, in some situations plants protected by dense bracken were damaged.

That damage to test plants was detected even at 25% of the full deposition rate is not surprising, because water sensitive papers is a crude method for detecting drift. Only large droplets are detected, as many of the finer droplets are below the resolution of this technique. These results, however, confirm those of Marrs *et al.* (1992) who detected deposition on water sensitive papers up to 30 - 40 m downwind of an aerial spray application but leaf damage to *R.acetosa* up to 161 m. Both studies show that where drift deposition on water sensitive papers can be detected, biological damage to sensitive species is likely to be considerable.

Both deposition and plant damage showed a concentration on the downwind edge of the clough, with effects being more pronounced on both the western clough side and the western top under dense bracken than on their eastern upwind counterparts. This result supports the suggestion of Marrs *et al.* (1992) that spraying should only be done downwind of sensitive sites, as this would enable buffer zone distances to be reduced. At present sensitive sites are surrounded by a 250 m buffer zone, although Marrs *et al.*

(1992) on the basis of a single experiment in unusually high wind speeds have suggested that 161 m is adequate. Clearly further information is required about the spray drift deposition around sensitive sites under a range of different spray application scenarios.

The methodology outlined in this paper is obviously sensitive enough to detect both the deposition of asulam drift and biological damage on test plants. As it has been used successfully in two other studies (Marrs *et al.*, 1990; Marrs *et al.*, 1992), we believe it is suitable (1) for improving spray application strategies around sensitive sites, and (2) for policing aerial applications of asulam in these situations (FEPA, 1989a,b).

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