PROGRESS REPORT ON BIOLOGICAL STUDIES OF FLUAZIFOP-P-BUTYL AND HALOXYFOP ETHOXYETHYL

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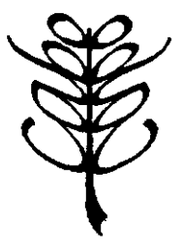

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Summary

In a germination test fluazifop-P-butyl and haloxyfop ethoxyethyl decreased the length of roots of barley, oats and wheat to more than a half of untreated controls even at low doses (0.65 and 0.325 mg/dm^3). With respect to the shoots, barley was more susceptible to the two herbicides than oats and wheat. The susceptibility of *Echinochloa crus-galli* and *Sorghum halepense* decreased at increasing age.

The effect was more pronounced after treatment of both roots and leaves than after treatment of the roots or the shoots separately. Incorporation of the herbicides in the soil, either mechanically or by rain, improved the pre-emergence activity. One hour after a post-emergence application, rain has no effect on the activity.

In humic sand the mobilities of fluazifop-P-butyl and haloxyfop ethoxyethyl were nearly the same and less than for atrazin. In sandy loam the mobility was nearly the same for the three herbicides.

For fluazifop-P-butyl the residual effects lasted less than 15 days in humic sand and for haloxyfop ethoxyethyl less than 30 days.

Samenvatting

In een kiemproef werd, zelfs bij lage concentraties (0,65 en 0,325 mg/dm^3) van fluazifop-P-butyl en haloxyfop ethoxyethyl, de lengte van de wortels van gerst, haver en tarwe meer dan gehalveerd in vergelijking met onbehandeld. De stengelscheuten reageerden bij gerst op beide middelen sterker dan die van haver en tarwe. De gevoeligheid voor de beide middelen nam af bij hanepoot en aleppogierst met het ouder worden van de planten.

Het effect van fluazifop-P-butyl en haloxyfop ethoxyethyl was bij een behandeling van zowel de wortels als de spruiten groter dan bij een behandeling van alleen de wortels of alleen de spruiten. Het inwerken van de middelen, hetzij mechanisch hetzij door inregenen, versterkte de werking bij een vóóropkomstbehandeling. Een beregening een uur na de toediening van de middelen had geen invloed meer op de werking.

In een humeuze zandgrond hadden fluazifop-P-butyl en haloxyfop ethoxyethyl ongeveer eenzelfde mobiliteit, maar een geringere dan atrazin. In een zavelgrond was de mobiliteit voor alle drie middelen ongeveer gelijk.

In een humeuze zandgrond kon fluazifop-P-butyl na 15 dagen niet meer worden aangetoond met tarwe en haloxyfop ethoxyethyl niet meer dan 30 dagen.

1. Introduction

During the winter of 1985/1986 a series of experiments were carried out under glasshouse conditions to obtain more information about the herbicides fluazifop-P-butyl (called Fusilade super) and haloxyfop ethoxyethyl (called Gallant 125 EE) on the following aspects:

a. the principles of their biological behaviour (phytotoxic activity, symptoms, uptake, translocation);
b. their general potential phytotoxicity and the specific sensitivity of some plant species;
c. their behaviour in the soil.

2. Plant growing techniques

Test plants used in the experiments were wheat (cv. Okapi), barley (cv.Grit), oats (cv. Condor), *Sorghum halepense* (L.)Pers. and *Echinochloa crus-galli* (L.)Beauv. These plants were cultivated by various methods, depending on the objectives of the experiments concerned. In the experiments the following variables were included:

a. the nature of the substrate;
b. the volume of the substrate per treatment;
c. the technique of adding moisture;
d. the species and/or growth stage of test plants.

2.1. Germination test

Method GT

- 15 seeds were placed in petri dishes (ϕ 9 cm) with a filter paper on the bottom;
- the filter paper was wetted with a solution of the herbicide;
- dishes were placed in a germination box at about 20 °C.

2.2. Growing test with plants in soil

Method GS 1

- plastic cups (150 cm^3) provided with cotton wool plugs through a hole in the bottom were filled with 200 g of dry soil;
- 12 seeds per cup were sown;

- wetting of soil by capillary action, by placing pots on plastic foam in shallow trays kept constantly wet.

Method GS 2

- plastic pots (Ø 9 cm) were provided with a filter paper on the bottom and were filled with 350 g of dry soil;
- a number of seeds of the test plants were spread over the soil surface;
- wetting of soil by capillary action, by placing pots on plastic foam in shallow trays kept constantly wet;
- after germination, the plants were thinned to 3 per pot.

2.3. Test plants grown in nutrient solution

Method NS

- seedlings were grown in pure sand and transplanted when they had well developed roots and a first leaf, not completely unrolled;
- plantlets were fixed between 2 plastic plant labels (padded with thin strips of plastic foam), fastened into vertical slits in the rim of 150 cm^3 plastic cups;
- usually 3 plants per cup were used and grown in a pure or herbicide-containing nutrient solution (Hoagland solution, half strength).

2.4. Supplementary details will be described together with the experiments concerned

3. Experiments and experimental methods

Throughout the experiments commercial products (emulgated concentrate with 125 g a.i./dm^3) were used. Dosages mentioned in this report are active ingredients, unless otherwise stated. Four replications were used in all studies.

A humic sand (org.matter 3.6%; pH-KCl 4.6) or a sandy loam (org.matter 2.3%; pH-KCl 3.7; lutum 7.2%; silt 39.8%) were used as a soil medium.

Experiment No. 1: Germination test

- concentrations of Fusilade super and Gallant 125 EE in water of 0; 0.325; 0.65; 1.3; 2.6 and 5.2 mg/dm^3 were added to the petri dishes according to method GT at 6.5 cm^3 solution per dish;
- test plants: wheat, barley and oats;
- duration of the experiment: 4 - 6 days.

Experiment No. 2: Dosage response in soils

- concentration series of the two herbicides were made in humic sand: 0; 0.025; 0.05; 0.1; 0.2; 0.4 and 0.8 mg/kg;
- growing technique GS 1;
- test plants: wheat, barley and oats;
- duration of the experiment: about 2 weeks.

Experiment No. 3: Dosage response in nutrient solution

- Concentration series were made in nutrient solution: 0; 0.05; 0.1; 0.2; 0.4; 0.8 and 1.6 mg/dm^3 of the two herbicides;
- growing technique: NS in 100 cm^3 cups;
- test plants: wheat, barley and oats;
- duration of the experiment: about 2 weeks.

Experiment No. 4: Different sites of application of Fusilade super and Gallant 125 EE

- growing technique: NS;
- dosages: 2 and 4 dm^3/ha of Fusilade super and Gallant 125 EE;
- treatments: leaves only, leaves and nutrient solution, and nutrient solution only;
- test plants: wheat, barley and oats;
- duration of the experiment: about 3 weeks;
- application: by a spraying machine with a boom with three nozzles, type Birchmeier Helico Sapphire 1.2-2F-0.6. The pressure was 2 kg/cm^2 and the sprayvolume 400 dm^3/ha;

- as the cups holding plantlets had no lids, the spraying boom passing overhead delivered the proportionate quantity of herbicide directly into the nutrient solution; for the leaves-only treatment the cups were temporarily covered.

Experiment No. 5: Post-emergence application of Fusilade super and Gallant 125 EE

- growing technique: GS 2;
- 0.75 and 1.5 dm^3/ha of Fusilade super and Gallant 125 EE were used either alone or with 3 dm^3/ha adjuvant - Ethokem or vegetable oil (rapeseed oil);
- test plants: *Sorghum halepense* and *Echinochloa crus-galli*;
- growth stage at the time of treatment was: 3; 4; 5 and 6-7 leaves;
- mode of application was the same as in experiment no.4;
- duration of experiment: about 4 weeks.

Experiment No. 6: Effect of rain on efficiency of herbicides

- growing technique GS 2;
- plants of *Sorghum halepense* were treated in the 3-leaf stage;
- just before spraying, *Echinochloa crus-galli* was sown in the same pots;
- 1.5 dm^3/ha of Fusilade super and Gallant 125 EE were sprayed in the same way as in experiment no.4;
- 1, 2 and 4 hours after herbicide treatment the plants were sprinkled by the same machine at a pressure of 0.6 kg/cm^2. The total volume of water used was 2.9 dm^3 (about 19 mm of rain).

Experiment No. 7: Soil incorporation studies

- growing technique: GS 1;
- dosages: 1.5 dm^3/ha of Fusilade super or Gallant 125 EE;
- incorporated mechanically to a depth of: 0; 2; 4; 6; 8; 10 and 15 cm, this means a concentration in the treated layer of 0; 0.71; 0.35; 0.23; 0.18; 0.14 and 0.09 mg/kg respectively;
- test plants: *Sorghum halepense* and *Echinochloa crus-galli*;

- depth of sowing: 1 cm;
- duration of the experiment: about 3 weeks.

Experiment No. 8: Mobility studies with Fusilade super, Gallant 125 EE and atrazin

- method described by Gerber, Ziegler and Dubach (1970);
- 1 cm^3 of a solution with 400 mg/dm^3 Fusilade super or Gallant 125 EE or with 4000 mg/dm^3 atrazin was dripped on the soil at about 1 cm from the topend of a slightly sloping tray covered with ½ cm of soil;
- water was percolated through the soil layer till 10 cm^3 had passed down the plate and fallen in a measuring glass at its end;
- after percolation, *Agrostis capillaris* L.(= *A.tenuis Sibth.*) was directly sown upon the soil layer;
- duration of the experiment: 2 - 3 weeks.

Experiment No. 9: Adsorption studies

- extent of herbicidebinding to soil was estimated by shaking herbicide-containing nutrient solution with humic sand, followed by growing test plants on the resulting solutions;
- proportion of soil/nutrient solution was 200 g/800 cm^3. The mixture was shaken for 48 hours and next filtrated;
- series A (extract of soil + herbicides + nutrient solution), starting concentrations of the solutions 0; 0.1; 0.3; 0.5; 1.0 and 2,0 mg/dm^3 of the two herbicides;
- series B (extract of soil + nutrient solution) as a basis for a standard series of the two herbicides with concentrations 0; 0,1; 0.15; 0.2; 0.4 and 0.8 mg/dm^3;
- growing technique: NS;
- test plant: barley;
- duration of the experiment: 2 weeks.

Experiment No. 10: Persistence of Fusilade super and Gallant 125 EE in soil

- growing technique: GS 1;
- test plant: wheat;

- soil treated with 0.5 mg/kg;
- duration of the experiment: 7 weeks;
- sowing was done on the day of treatment and 5, 10, 15, 20 and 30 days later.

Experiment No. 11: Effect of Fusilade super and Gallant 125 EE on uptake of $^{14}CO_2$ and on translocation of assimilates in plants

- growing technique: NS;
- test plant: oats;
- plants were treated via the leaves with the spraying machine mentioned, dosage 1.5 dm^3/ha of the two herbicides; and via the roots by growing the plants in a nutrient solution, herbicide concentration 0.4 mg/dm^3;
- treatments with herbicides took place three days before exposure to $^{14}CO_2$;
- exposure to $^{14}CO_2$ for 5 hours, after which the plants remained for 24 hours in a climate room to translocate the assimilates;
- then the plants were pressed and dried;
- dried plants were exposed to X-ray film for 10 days.

4. Results

4.1. Germination test with Fusilade super and Gallant 125 EE (Exp. 1)

The germination of the seeds itself was not influenced by the herbicides, but there was a considerable effect on the growth of young roots of wheat, barley and oats. In comparison to untreated controls, the length of roots was more than halved, even at the lowest rate. With respect to the shoots the situation was somewhat different. Barley was more susceptible to the two herbicides than wheat and oats.

In comparison with other growth inhibitors, the inhibiting effect was considerable, since shoots and roots were already inhibited at 0.3 mg/dm^3 (ethofumesate inhibition started from 2 mg/dm^3 in oats; Ostojić and Reisler, 1973).

When we compare wheat, barley and oats we may conclude that wheat was less susceptible than the other two species. When we compare

Fusilade super and Gallant 125 EE it was apparent that the former herbicide had somewhat more effect than the latter except in oats (Fig. 1).

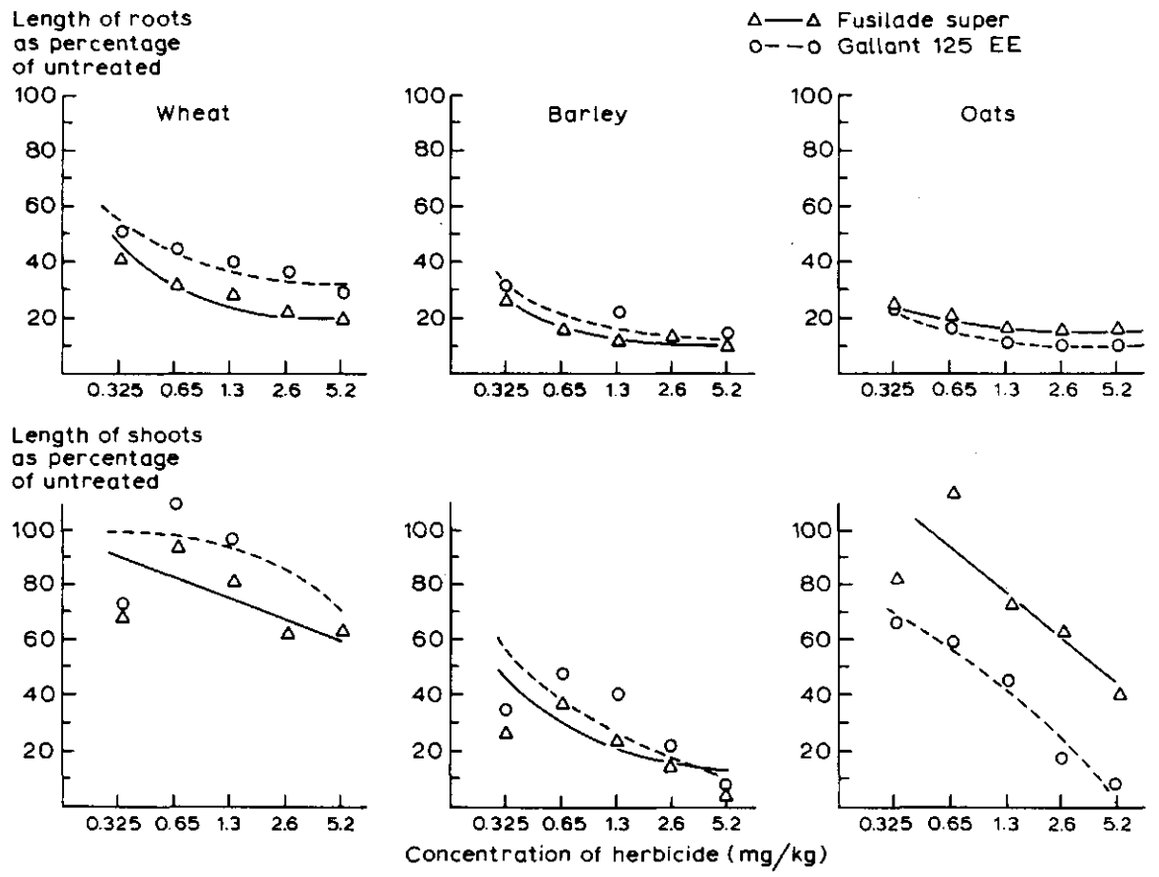

Fig. 1. Relative length of roots (above) and shoots (below) of wheat, barley and oats in relation to herbicide concentration in a test on filter paper.

4.2. Dosage response of plants grown in sandy soil (Exp. 2) or nutrient solution (Exp. 3) with herbicides

As in the previous trial, both herbicides had a negative effect on the test plants. Fusilade super from 0.2 mg/dm^3 caused heavy growth inhibition of wheat, barley and oats. Gallant 125 EE did so from 0.1 mg/dm^3, except in barley (Fig. 2). Low concentrations (under 0.1 mg/dm^3) mostly stimulated growth. Growth inhibition occurred within 48 hours, which means that uptake of the herbicides by the roots and their translocation to the meristematic tissue is very fast.

In contrast with section 4.1, in this experiment oats was the most susceptible, barley the least. The results in nutrient solution and soil were similar, but for the same effect in soil a higher dosage was needed (compare Fig. 2 with Fig. 3).

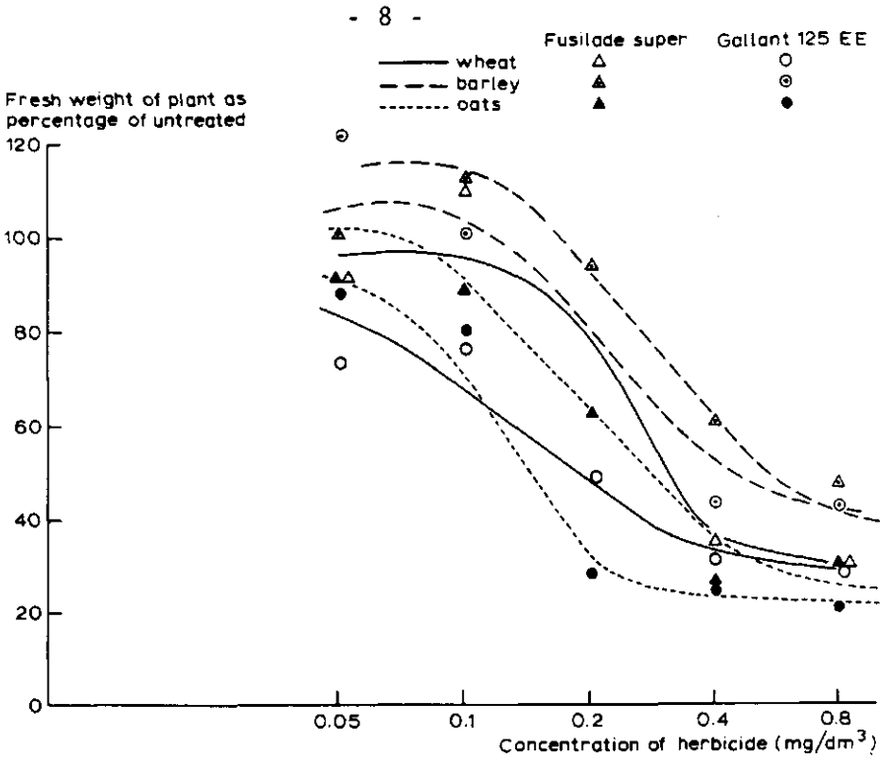

Fig. 2. Effect of concentration of Fusilade super or Gallant 125 EE in a nutrient solution on the relative fresh weight of whole plants of wheat, barley and oats.

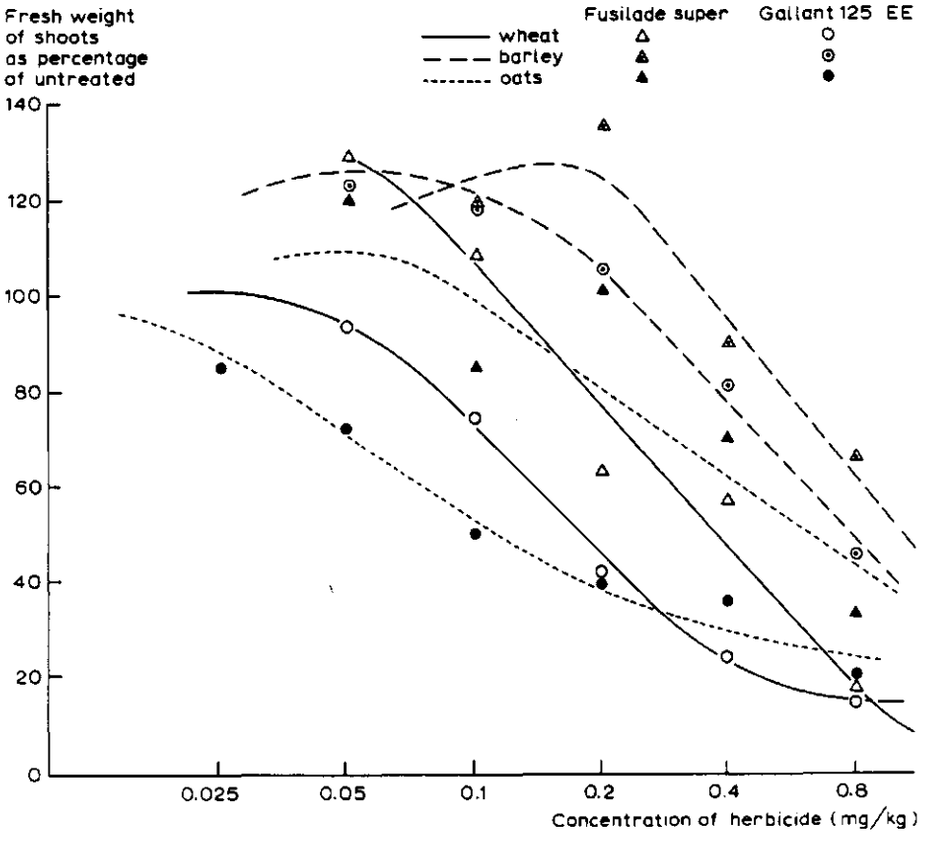

Fig. 3. Effect of concentration of Fusilade super or Gallant 125 EE in a humic sand on the relative fresh weight of shoots of wheat, barley and oats.

4.3. Uptake of the herbicides by the roots and the shoots (Exp. 4)

This experiment was intended to assess the influence of Fusilade super and Gallant 125 EE applied in various ways at the same growth stage. Test plants treated via the roots, roots and leaves, and only via the leaves showed different susceptibility.
Treatment of plants via both roots and leaves was more effective than treatment of the roots and of the leaves separately.

Of the test plants again oats was the most susceptible, but now wheat the least (Table 1).

Table 1. Activity of Fusilade super and Gallant 125 EE with different sites of application.

Sites of application	Dosage(dm^3/ha)											
	Fusilade super						Gallant 125 EE					
	wheat		barley		oats		wheat		barley		oats	
	2	4	2	4	2	4	2	4	2	4	2	4
Roots+leaves	3-4	3	2-3	2	2	1-2	3	2-3	2-3	2	1-2	1
Leaves	5	4	4-5	3-4	3	2-3	4	3-4	3-4	2-3	2-3	2-3
Roots	7	6	5	4-5	4-5	4	6	5-6	4-5	4	4	4

1=plants killed 2-5=necrosis 6-9=yellowing 10=control

Oats treated with Gallant 125 EE was killed in about 7 days by root-and-leaf application, in 10 days by leaf application and in 15 days by root application. With the same ways of application the barley plants were killed from 5-7 days later.

Comparing Fusilade super and Gallant 125 EE, the former herbicide had a slower action and needed 5-7 days longer for the same effect.

The first symptoms caused by the herbicides, yellowing of the basis of the youngest leaf, were independent of the way of application.

Generally, killing of the plants depended on the mode of application of the herbicides and on the type of herbicide.

4.4. Post-emergence application (Exp. 5)

This experiment was intended to study the susceptibility of some weed species, as depending on their age, rate of application and adding of adjuvants.

Test plants were grown according to technique GS 2 and next sprayed with Fusilade super and Gallant 125 EE.

Of the two weed species, *Sorghum halepense* showed the highest and *Echinochloa crus-galli* the lowest susceptibility. Susceptibility decreased from young to older plants. For each additional leaf the time needed to kill the plants was prolonged by 5-7 days (Table 2).

Table 2. Effect of Fusilade super and Gallant 125 EE on *Sorghum halepense* and *Echinochloa crus-galli* in different stages of development, 15 days after treatment.

Herbicides	*Sorghum halepense*				*Echinochloa crus-galli*			
	number of leaves				number of leaves			
	3	4	5	6	3	4	5	6
Fusilade super	2	3	4	6-7	2	3	4-5	7
Gallant 125 EE	1	2	3	6	1	2-3	3-4	7

1= totally killed 10= healthy

Speed of action of Fusilade super was much slower than that of Gallant 125 EE; this could be concluded from the estimates of the effect of the herbicides in the different leaf stages. For example, Fusilade super applied in the three leaf stage showed the same effect as Gallant 125 EE in the four leaf stage. The first symptoms after foliar application occurred after three days as an interruption of growth, yellowing of the basis of the youngest leaf and leaf desication beginning at the tip. We did not observe better action of the herbicides when Ethokem or vegetable oil were added, but with Ethokem we obtained distinct necrotic spots on the treated leaves.

4.5. Effect of rain (Exp. 6)

In this experiment it was confirmed that one hour after a post-emergence application of these herbicides, rain has no influence on their activity (Plowman et al., 1980; Anonymus 1981).

Herbicides can be incorporated in the soil by rain, so enabling pre-emergence activity of the herbicide; in this trial *Echinochloa crus-galli* did not emerge. After spraying the same concentrations of the herbicides in the same way, but not followed by artificial rain, *Echinochloa crus-galli* emerged and grew well.

4.6. Incorporation studies (Exp. 7)

The study was intended to assess the influence of Fusilade super and Gallant 125 EE incorporated to different depths in a humic sandy soil (decrease of the starting concentration with increase of the thickness of the treated layer), on development of *Sorghum halepense* and *Echinochloa crus-galli*.

The results show that the herbicides generally had much effect on the growth of the plants of both test species which were heavily suppressed. Only when Fusilade super was incorporated to 15 cm depth the effect was relatively poor (Fig.4).

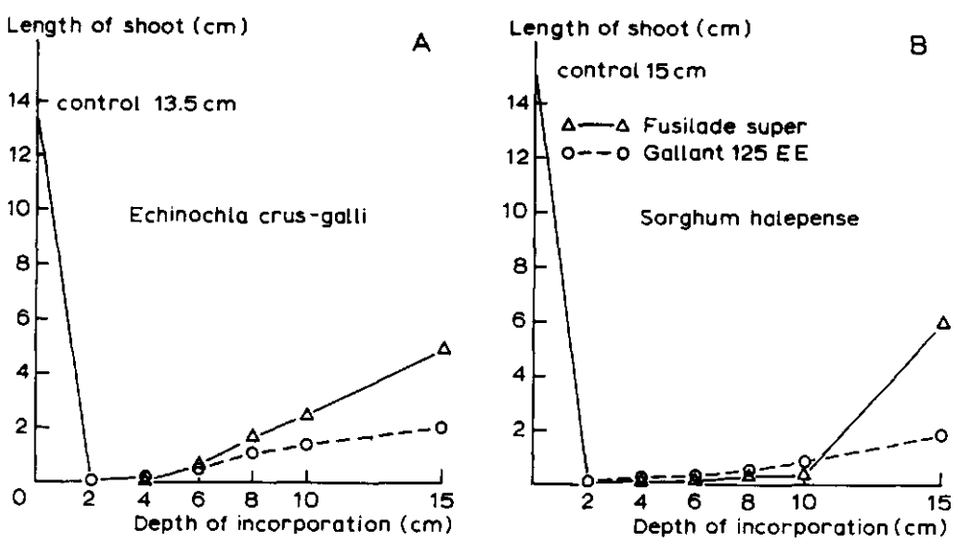

Fig. 4. Effect of depth of incorporation in a humic sand of Fusilade Super or Gallant 125 EE (1.5 dm^3/ha) on the length of shoots of *Echinochloa crus-galli* (A) and *Sorghum halepense* (B).

4.7. Mobility in soil (Exp. 8)

Methods to measure mobility were described by Gerber et al. (1970) and were used to compare the mobility of Fusilade super, Gallant 125 EE and atrazin in humic sand and sandy loam. The results show that Fusilade super and Gallant 125 EE have a much lower mobility than atrazin in humic sand (3-4 cm). In the sandy loam mobility of the three herbicides was nearly the same and much higher than in humic sand (Table 3).

Table 3. Leaching in soil in cm, as evidenced by growth suppression of *Agrostis capillaris*.

Herbicides	Humic sand		Sandy loam	
	leaching max. till	highest concentration at	leaching max. till	highest concentration at
Fusilade super	3-4	2-3	20	0-13
Gallant 125 EE	3-4	2-3	20	0-13
Atrazin	9-11	8-10	20	4-14

4.8. Adsorption in soil (Exp. 9)

The concentration of the herbicides in the filtrate after shaking was estimated with the help of Figs. 5 and 6. The constants of adsorption were measured graphically and were about 10 kg/dm^3 for humic sand (Fig.7). In a comparable humic sand soil the adsorption constant was 22 kg/dm^3 for diuron and 6.5 kg/dm^3 for ethofumesate (Ostojic and Reisler, 1973), so the adsorption of Fusilade super and Gallant 125 EE is high.

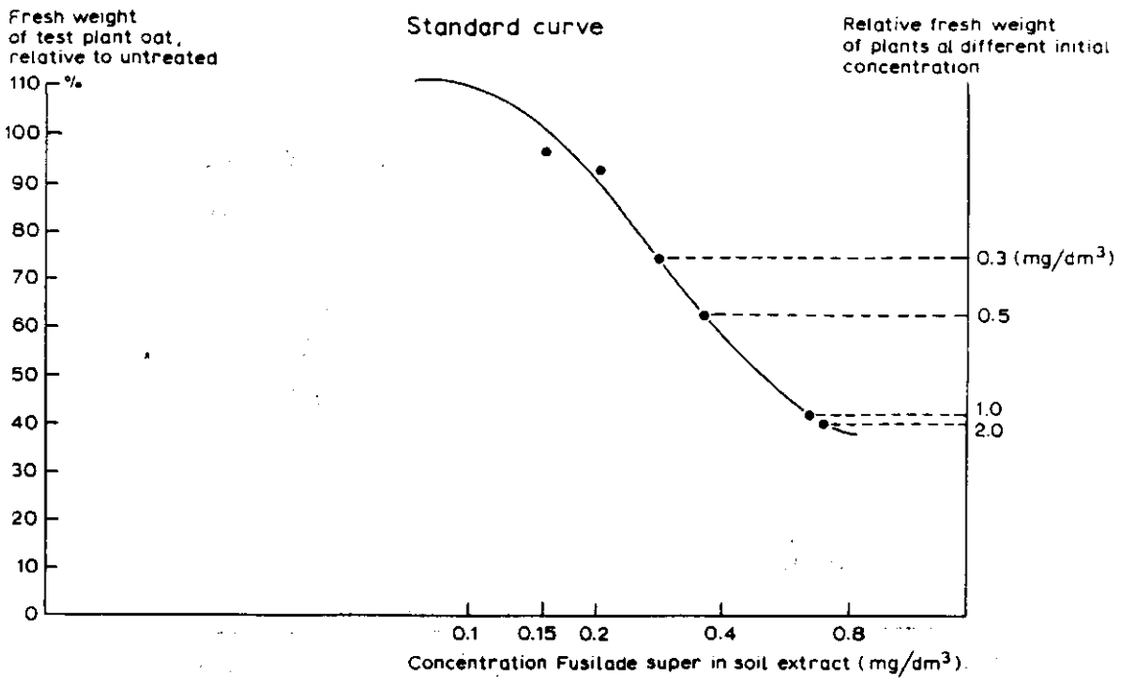

Fig. 5. Effect of concentration of Fusilade super in an extract of humic sand on the relative fresh weight of shoots of oats. At the right side the estimated concentration after interpolation of the relative weight of oats grown on the filtrate.

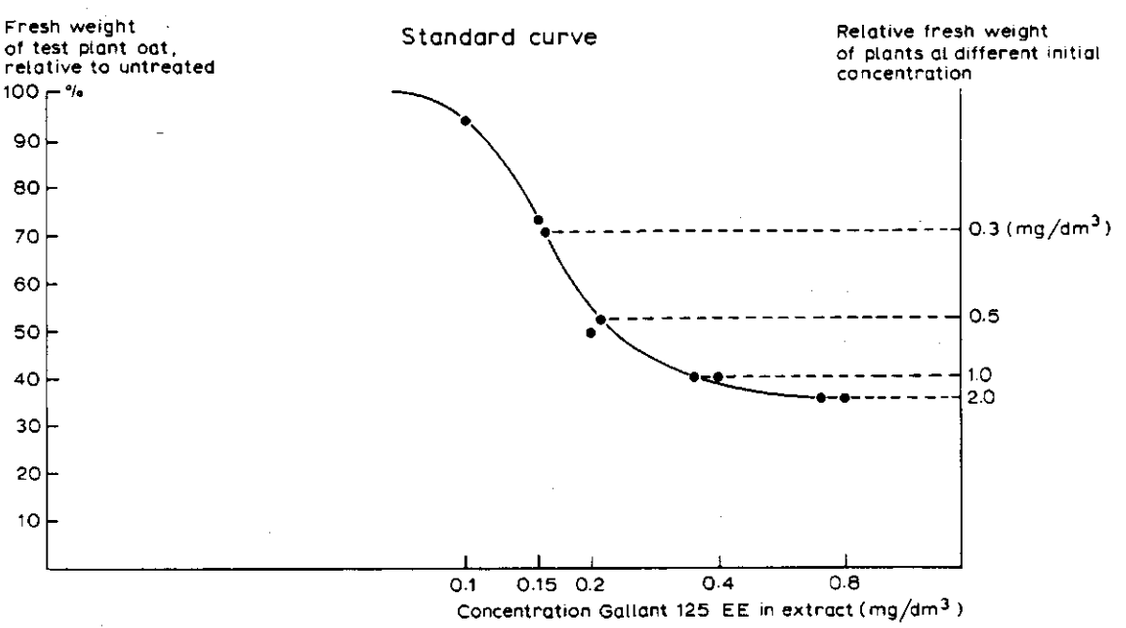

Fig. 6. Effect of concentration of Gallant 125 EE in an extract of humic sand on the relative fresh weight of shoots of oats. At the right side the estimated concentration after interpolation of the relative weight of oats grown on the filtrate.

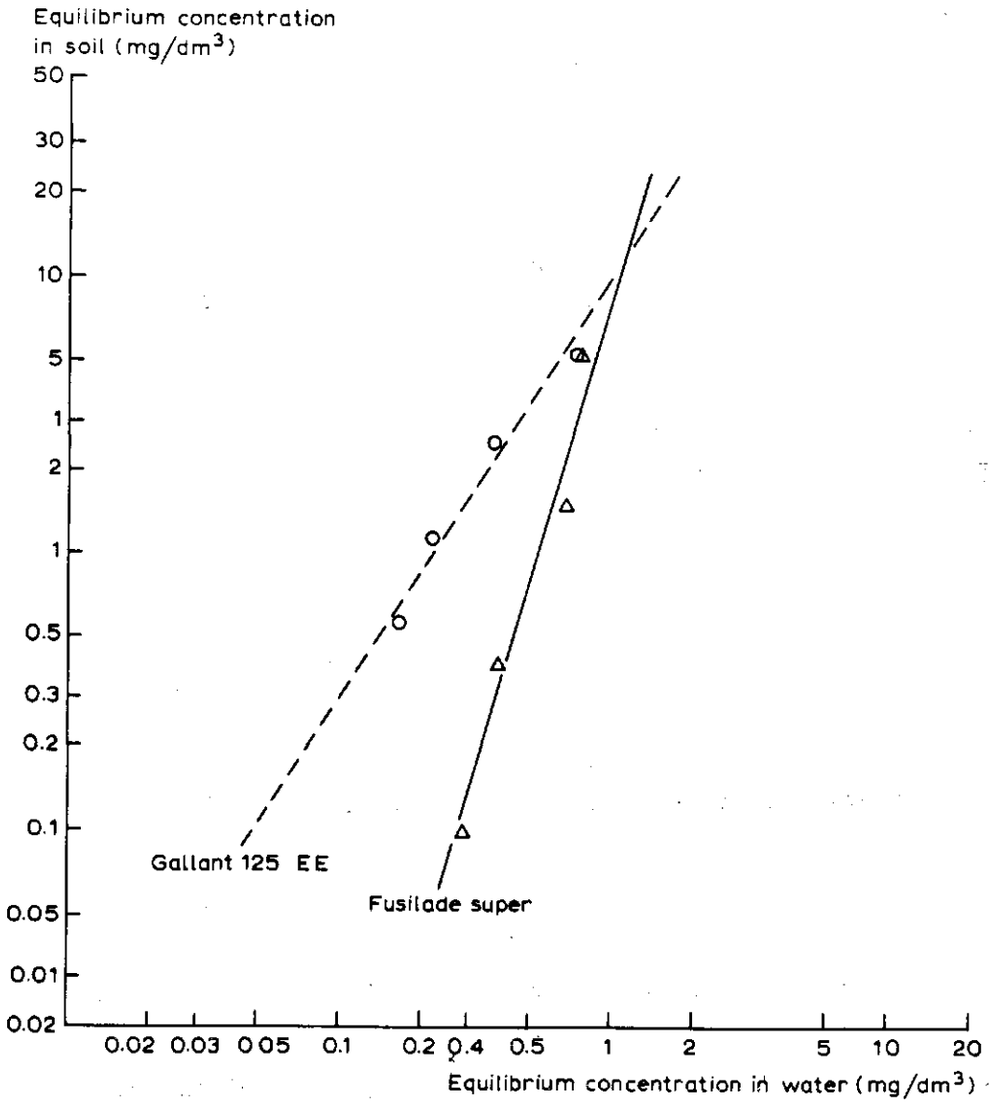

Fig. 7. Isotherms for the adsorption of Fusilade super and Gallant 125 EE by a humic sandy soil.

4.9. Persistence of Fusilade super and Gallant 125 EE in soil (Exp. 10)

Because these herbicides showed pre-emergence activity, the purpose of this study was to estimate the duration of this activity in humic sand. From the results it may be concluded that Fusilade super remains active in the soil for about two weeks, whereas Gallant 125 EE was active even after three weeks. But after 30 days the effect of Gallant 125 EE on development of the test plants was negligible (Fig.8).

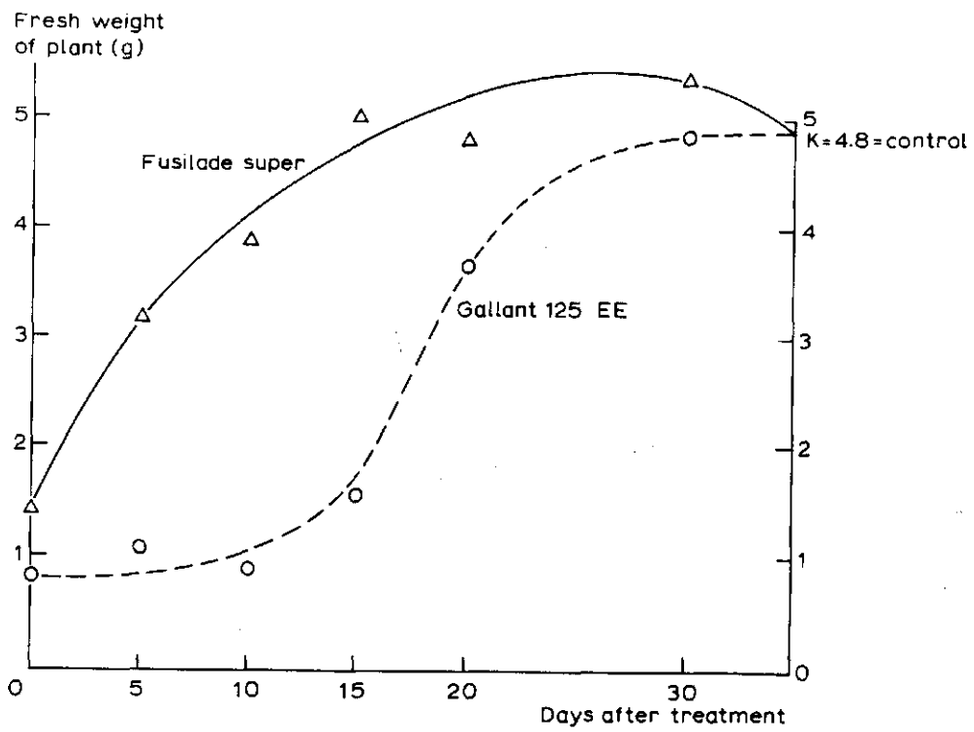

Fig. 8. Fresh weights of sprouts of wheat in relation to the persistance of Fusilade super and Gallant 125 EE.

4.10. Effect on uptake of $^{14}CO_2$ and translocation of assimilates (Exp. 11)

An impression of the $^{14}CO_2$ uptake and distribution of assimilates in the plant was obtained by radio-autography. Weak radioactivity was observed in young leaves and the meristematic tissue of the plants treated with herbicides. Herbicides accumulating in meristemic tissue apparently cause such changes that this part of the plant can no longer accumulate assimilates.

5. Characteristics of Fusilade super and Gallant 125 EE

- Both herbicides showed great activity as inhibitors of root growth even at low doses (0.65 and 0.325 mg/dm^3). With barley there was nearly the same effect on roots as on shoots (Fig. 1).
- At low rates (0.1 - 0.2 mg/dm^3 or kg) in nutrient solution and soil the herbicides depressed growth of the plants. Consequently uptake by the roots is important (Fig. 2 and 3).