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STØTTET AF

# Promilleafgiftsfonden for landbrug

## VIII Influence of weed growth stage and moisture stress on the efficacy of glyphosate

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In Denmark few herbicides are available for weed control in potatoes, with only pre-emergence herbicides authorised for broadleaf weed control. Glyphosate in a tank mix with a residual herbicide is often used pre-emergence to control early flushes of weeds. However, reports from potato growers claim that the efficacy of glyphosate is variable. Two plausible explanations for differences in the susceptibility of weeds to glyphosate were tested: 1) different growth stages and 2) drought stress. The results indicate that differences in growth stages at the early development stages can explain some of the variability in the efficacy of glyphosate in potatoes, for example we found a 2.5 to 3.8-fold increase in the ED<sub>90</sub> doses at BBCH 12-13 compared to the cotyledon stage. Responses to moisture stress was measured in four experiments with one significant response, a tendency to reduced activity found in two experiments and one experiment with no change. The results indicate that severe moisture stress at spraying can - but does not necessarily - have an adverse effect on glyphosate activity.

Weed control in potatoes relies on a small number of herbicides with the herbicides used for broadleaf weed control only authorised for pre-emergence application. Early flushes of weeds are often controlled with glyphosate in combination with a residual herbicide pre-emergence of the crop. Reports from the potato growers claim that the efficacy is variable. Although the weeds are quite small at the time of application, differences in the susceptibility at these early growth stages may account - at least partly - for these variable effects. Another factor that might play a role is the soil moisture. Potatoes are cultivated on sandy soils, and the mechanical soil cultivation carried out prior to planting reduces moisture stored in the upper soil layer. It is well known that herbicide performance is generally reduced on moisture-stressed plants.

The objectives of these experiments were to examine: 1) the influence of weed growth stage and 2) the influence of moisture stress on the efficacy of Roundup Flex (480 g/L glyphosate). The experiments were carried out on two weed species commonly found in potato fields: oilseed rape (*Brassica napus*) and black bindweed (*Polygonum convolvulus*).

#### Material and methods

#### The influence of growth stage

To examine the effect of growth stage in oilseed rape, seeds were sown in 2-L pots in a potting mixture consisting of sand, soil and peat including all necessary micro- and macronutrients. Groups of pots were sown at 4 to 5-day intervals in order to obtain plants with different growth stages which could all be sprayed on the same day. After seedling emergence, the number of plants per pot was reduced to four. A similar experiment was conducted on black bindweed. The germination of seeds of this species is often low and uneven, and in order to generate uniform plants the seeds were sown in trays in the glasshouse and the seedlings transplanted to 2-L pots (3 plants per pot). This procedure was also repeated at 4 to 5-day intervals in order to obtain groups of plants with different growth stages. All plants were grown outdoors.

The two experiments with oilseed rape were sprayed on 2 July (experiment 1) and 3 August (experiment 2); the experiment with black bindweed was sprayed on 26 June (experiment 3). Herbicide application

was carried out in a spray cabinet. Each treatment was applied at six to eight doses in a spray volume of 160 to 180 L/ha. The doses on oilseed rape ranked from 7.5 to 720 g/ha and on black bindweed from 7.5 to 240 g/ha with three replicates per treatment.

#### The influence of soil moisture

The experiments examining the influence of moisture stress were conducted on oilseed rape (2 experiments) and black bindweed (2 experiments). Both species were established as described above. The pots were watered from below until the cotyledon stage. Then moisture stress was induced by turning off the water supply for groups of pots. At the same time the number of plants per pot was reduced to three. All test plants were grown outdoors.

The moisture stress experiments on oilseed rape were sprayed at the 2-4 leaf stage (experiment 4 on 26 June and experiment 5 on 6 July). The black bindweed was sprayed at the 2-3 leaf stage (experiment 6 on 6 July and experiment 7 on 17 August). In each of the experiments six doses of glyphosate were applied, ranking from 11.3 to 720 g/ha. Watering of the moisture-stressed plants was restarted three days after herbicide application.

The plants from all the experiments were harvested two to four weeks after spraying. Fresh and dry weights were recorded. A dose-response model was fitted to the data and  $ED_{00}$  doses were estimated.

#### Influence of growth stages

The growth stages of oilseed rape varied from BBCH 10 (cotyledon stage) to BBCH 14 (4 leaf stage) at spraying (experiments 1 and 2) while the growth stages of black bindweed in experiment 3 varied from BBCH 10 to BBCH 12 (2 leaf stage) at spraying. Photos of the weeds prior to spraying are shown in Figures 1 and 2.



**Figure 1.** Growth stages of oilseed rape at spraying (experiment 1). From left to right: BBCH 10, BBCH 11.5, BBCH 12, BBCH 13.5 and BBCH 14.



**Figure 2.** Growth stages of black bindweed at spraying (experiment 3). From left to right: BBCH 10, BBCH 10.3 and BBCH 12-13.

The susceptibility of oilseed rape to glyphosate was similar across the two spraying dates except for the cotyledon stage for which the  $ED_{90}$  was 12 g/ha glyphosate in experiment 1 and 22.9 g/ha in experiment 2 (Table 1). In both trials the plants were significantly more tolerant to glyphosate after the 2 leaf stage (>BBCH 12) compared to the cotyledon stage with dose requirements for a 90% reduction of biomass increasing by a factor 3.8 in experiment 1 and 2.4 in experiment 2. A very large increase in  $ED_{90}$  at BBCH 14 was observed in experiment 1. On black bindweed, a 3-fold increase in the  $ED_{90}$  dose was required when plants developed from the cotyledon stage to the 2-3 leaf stage. Overall, the  $ED_{90}$  doses for both weed species were quite low as is often seen in pot experiments. The required doses in the field are higher but experience from previous experiments shows that the ratio between dose requirements at the different growth stages can be transferred to the field. These results indicate that differences in growth stages at the early development stages can explain some of the variability in the efficacy of glyphosate in potatoes as exemplified by the 2.5 to 3.8-fold increases in the  $ED_{90}$  doses at BBCH 12-13 compared to the cotyledon stage for both weed species.

**Table 1.** Estimated  $ED_{90}$  doses (g/ha) of glyphosate applied at different growth stages of oilseed rape and black bindweed. Brackets show 95% confidence intervals. N.d.= not determined.

Growth stage	Oilseed rape		Black bindweed
	Experiment 1	Experiment 2	Experiment 3
BBCH 10	12.0 (10.1-13.9)	22.9 (19.4-26.4)	34.9 (7.6-42.1)
BBCH 10.3	n.d.	n.d.	34.5 (25.7-43.3)
BBCH 11.5	33.0 (27.2-38.9)	26.6 (23.1-30.0)	n.d.
BBCH 12	35.7 (29.1-42.3)	38.9 (34.0-43.9)	107.4 (98.0-116.8)
BBCH 13.5	45.2 (35.5-54.9)	54.3 (46.8-61.7)	n.d.
BBCH 14	167.1 (112.5-221.7)	n.d.	n.d.

#### Influence of soil moisture

The oilseed rape grown at low soil moisture suffered severely from moisture stress at spraying. At the time of application the leaf area of the moisture-stressed plants was reduced by 73% and their fresh weight was reduced by 78%. The leaves of plants growing at low soil moisture were more upright and the colour of the true leaves was darker green. Photos of the oilseed rape plants are shown in Figure 3.



**Figure 3.** Oilseed rape growing at optimum (left) and low (right) soil moisture conditions (experiment 4).

**Table 2.** Estimated  $ED_{90}$  doses (g/ha) of glyphosate on oilseed rape growing at optimum or low soil moisture. Brackets show 95% confidence intervals. N.d.= not determined.

Soil moisture	Experiment 4 BBCH 12	Experiment 5 BBCH 13-14
Optimum	184.7 (144.8-224.6)	226.0 (165.9-286.1)
Low	228.9 (181.1-275.9)	346.0 (275.8-416.3)
Low, water applied 4 hours after herbicide application	157.5 (126.2-188.8)	n.d.

The dose demand was higher on oilseed rape growing at low soil moisture levels compared to plants well supplied with soil moisture. The  $ED_{90}$  doses increased by a factor 1.2 and 1.5 in experiments 4 and 5 respectively, but the differences were not significant (Table 2). In experiment 4, a group of plants suffering from low soil moisture at spraying were supplied with water a few hours after spraying, and the response of these plants to glyphosate was apparently similar to the response of plants growing at optimum moisture conditions during the entire experiment.

The results of moisture stress on black bindweed varied in the two experiments with no differences in responses to glyphosate on optimally watered and drought-stressed plants in experiment 6 and a significant reduced effect of glyphosate on plants growing at low soil moisture in experiment 7 (Table 3).

In general, plants growing under low soil moisture conditions have smaller leaves, develop thicker cuticles and excrete more wax than plants grown under adequate moisture conditions. Such changes in size and surface characteristics may influence both herbicide retention and uptake. In addition, low soil moisture can also reduce the translocation of herbicide within the plants. In this study, a significant response to low soil moisture was found in experiment 7, a tendency to reduced activity in two experiments (experiments 4 and 5) and no change in response was found in experiment 6. The results indicate that severe moisture stress at spraying can - but does not necessarily - have an adverse effect on glyphosate activity.

**Table 3.** Estimated  $ED_{90}$  doses (g/ha) of glyphosate on black bindweed growing at optimum or low soil moisture. Brackets show 95% confidence intervals.

Water status	Experiment 6 BBCH 13	Experiment 7 BBCH 12
Optimum	137.5 (79.6-195.3)	43.3 (35.9-50.6)
Low	118.1 (70.4-165.8)	87.0 (69.3-104.7)

In conclusion, these results support that growth stages and moisture stress can have an effect on glyphosate performance; however, these factors alone are considered insufficient to account for the variability seen in potato fields across Denmark.

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