



## Relative effects of pre-treatment of ethephon, glyphosate and paraquat on glyphosate translocation and potency in control

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### ABSTRACT

Experiments were conducted to standardize duration and level of senescence required to enhance translocation of glyphosate in *Cyperus rotundus*. Senescence was induced by paraquat (500 g/ha) or glyphosate (1.312 kg/ha). Decrease in total chlorophyll and membrane damage was more in herbicide treatments than ethephon. Periodic leaf RWC remained unchanged in control but decreased periodically in ethephon and paraquat. Significantly higher RWC was observed at 72 h in glyphosate than paraquat. Maintenance of membrane integrity and high RWC in glyphosate at 72 h than paraquat facilitated mobility of glyphosate. Total biomass reduction bioassay indicated that senescence induced by glyphosate (1.312 kg/ha) pre-treatment 48 h followed by (*fb*) glyphosate (1.312 kg/ha) showed significantly more efficacy (7%) than pre-treatment with benzyl adenine (synthetic cytokinin) *fb* glyphosate which reduced efficacy by 9% compared to glyphosate alone.

**Key words:** Glyphosate, Herbicide translocation, Leaf senescence, Nutrient scavenge

Leaf senescence is a highly regulated process involving senescence associated genes which degrades chloroplasts followed by lipid degradation and nucleic acid. Ethephon produces ethylene, a senescence hormone. Paraquat inhibits photosynthesis, accepts electrons and transfers them to molecular oxygen, produce reactive oxygen species (ROS) leading to membrane damage. Experiments were carried out to assess the role of senescence induced by ethylene or herbicides on translocation and efficacy of glyphosate as reflected by biomass reduction bioassay, and herbicide induced senescence was compared with ethephon induced senescence on leaf physiology of *Cyperus rotundus*.

### MATERIALS AND METHODS

Experiments were conducted to standardize duration and level of senescence required to enhance translocation of glyphosate and to assess role of herbicide induced senescence on leaf physiology. Medium size (2 g/tuber) single tuber of *C. rotundus* per pot were sown in pots filled with soil : sand : FYM (6:3:1) mixture. Pots were irrigated as and when required. Forty-five days old plants were used for senescence experiments. The best duration of senescence was assessed by spraying glyphosate (1.312 kg/ha) and allowed to senescence for varied durations (0, 24, 48, 72 and 15 days), *fb* different glyphosate concentration, *viz.* 0, 0.33, 0.66, 0.984 and 1.31 kg/ha for each set

of varied duration of senescence. After 45 days of first spray, total biomass of *C. rotundus* response (logit) was regressed to varied concentration of glyphosate.

After identifying the best senescence induction duration (48 h), the level of glyphosate required for senescence for better control of *C. rotundus* was standardized. Senescence induced by varied glyphosate dosages (0, 0.32, 0.65, 0.98 and 1.31 kg/ha) for 48 h followed by same glyphosate dosages was sprayed to each set of varied level of senescent plant. After 45 days from second spray, the total biomass was recorded and logit response of different level of senescence to varied concentration of glyphosate was assessed. The total numbers of pots were 75 with three replications for each treatment combination for both level and duration experiments.

Mean and standard deviation of percent biomass (g/plant) over control was computed and converted into logit value. Logit (eq. 1) response was regressed with logarithmic concentration of glyphosate. Thus, dose-response regression lines were obtained with goodness of fitness (Devendra *et al.* 1997). Based on shift of regression line best duration and level of senescence has been estimated.

Logit  $Y = a + b (\log_e(X)) - (1)$ , where Logit  $Y = \{(D - Y/Y - C)\}$ , D and C indicate maximum (100) and minimum (0) per cent response over control, Y denotes percentage of observed biomass over control while a and b represent intercept and slope of regression line and X is the log<sub>e</sub> concentration of glyphosate (g/ha).

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To 45 days old *C. rotundus* induced senescence by spraying glyphosate (1.312 kg/ha), or paraquat (500 g/ha) and ethephon (5000 ppm) leaf chlorophyll content, membrane integrity and RWC was assessed at Periodic 24, 48 and 72 h.

Fresh leaf samples were collected and kept immersed in acetone and dimethyl sulphoxide (DMSO) of 1:1 proportion for 24 h in vacuum, filtered and optical density of the extract was measured at 652 nm (Hiscox and Israelstam 1979) and expressed as mg/g (eq.1).

Total chlorophyll (mg/g) = OD at 652 x (volume made up/1000) x 1/fresh weight (g) ..... (1)

Cell membrane integrity was measured by leakage of electrolyte (Sullivan and Ross 1979). Ten leaf dishes from periodic fresh leaf samples were incubated in 10 ml of distilled water for 2 h. Leakage of electrolyte into decanted water was measured at 273 nm (initial OD). Water was transferred back and respective leaf discs was boiled for 7 M, cooled and filtered and leakage of electrolyte into decanted water was measured at 273 nm (final OD) and expressed as per cent (eq. 2).

Per cent leakage = (initial OD/final OD) x 100 ..... (2)

Periodic fresh leaves were collected and fresh weight was recorded, then leaves were transferred to petri-dish containing water. leaves were allowed to imbibe water for 4 h and than recorded turgid weight. The leaves were oven dried at 80°C for 48 h, weighted and RWC were estimated using eq. 3 (Barrs and Weatherly 1962).

RWC = (fresh weight - dry weight)/(turgid weight - dry weight) x 100 ..... (3)

## RESULTS AND DISCUSSION

Regression analysis showed that glyphosate induced senescence at 48 h had logit biomass to glyphosate and response regression line drastically reduced compared to other duration of senescence (Fig. 1). The slope between different durations regression lines was not same and biomass was drastically reduced for all glyphosate induced regressions compared to non-senescent (0 glyphosate regression). Highest slope of 1.08 was noticed by glyphosate (1.31 kg/ha) induced senescence.

The logit biomass - glyphosate (1.31 kg/ha) at 48 h level of senescence regression line separated out with other levels of glyphosate regression lines (Fig. 2) and the shift was non-parallel, indicating that senescence induced 48 h period responded drastic reduction of biomass for glyphosate of 1.31 kg/ha and more superior to lower dose of glyphosate.

Total chlorophyll content drastically reduced in herbicidest than ethephon - natural senescence inducer (Fig. 3). Total chlorophyll content at 24 h of ethephon treated leaf was on par with control and 72 h later less than control but more than glyphosate and paraquat. This indicated that ethephon induced senescence was superior to glyphosate and paraquat with intact chlorophyll thus has higher energy for scavenging.

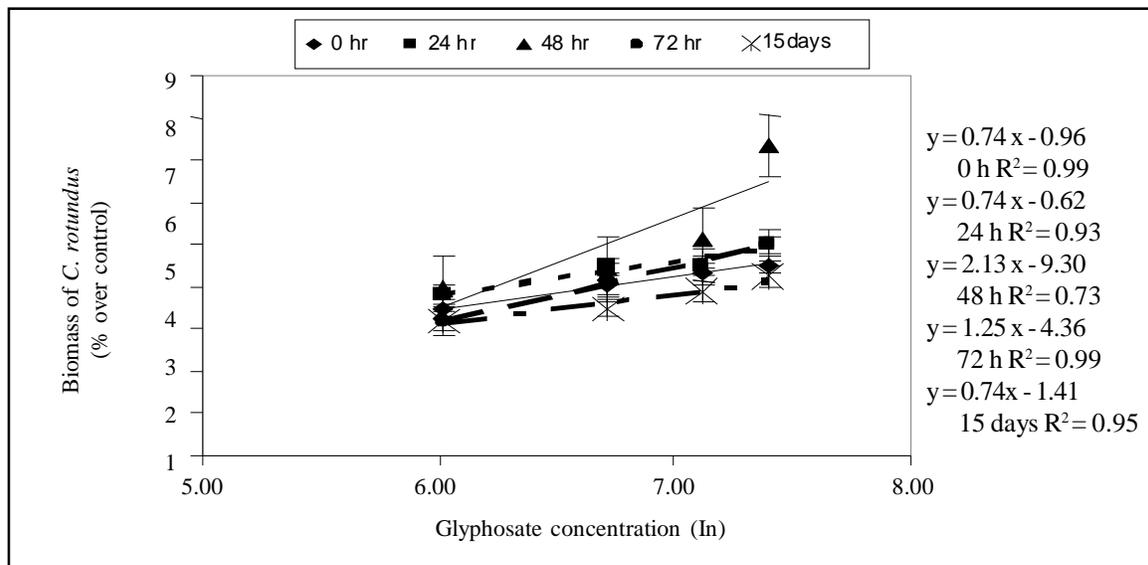


Fig. 1. Effect of varied period of senescence by glyphosate on biomass of *Cyperus rotundus* (error bar indicates replication variation)

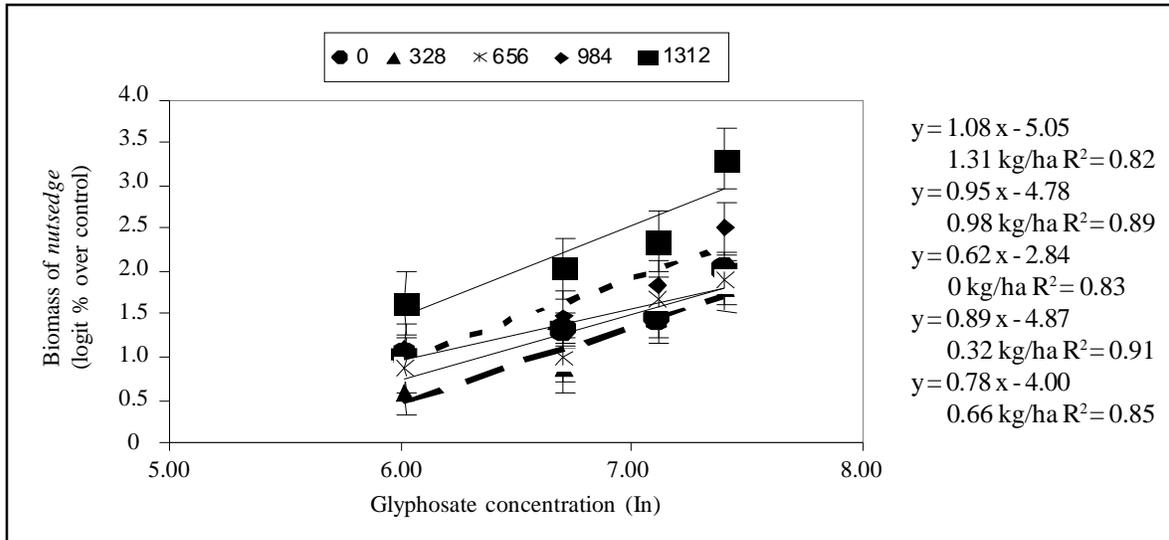


Fig. 2. Effect of level of glyphosate induced senescence for 48 h on biomass of *Cyperus rotundus* (error bar indicates replication variation)

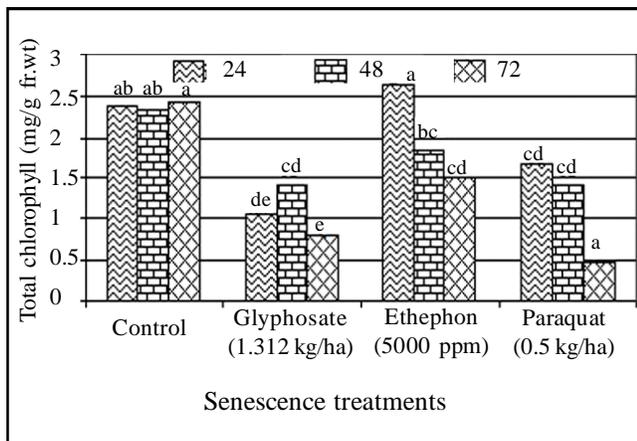


Fig. 3. Effect of glyphosate, paraquat and ethephon on total chlorophyll content in *Cyperus rotundus*

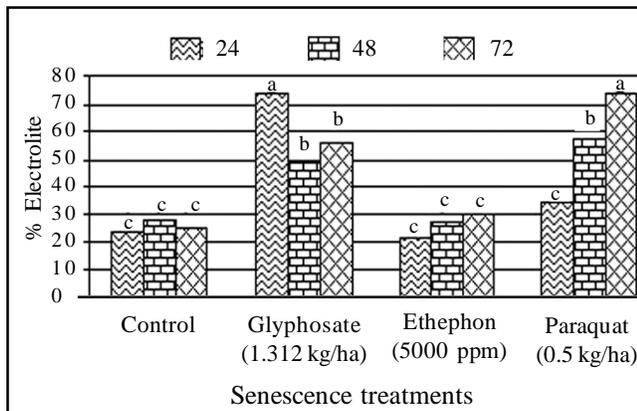
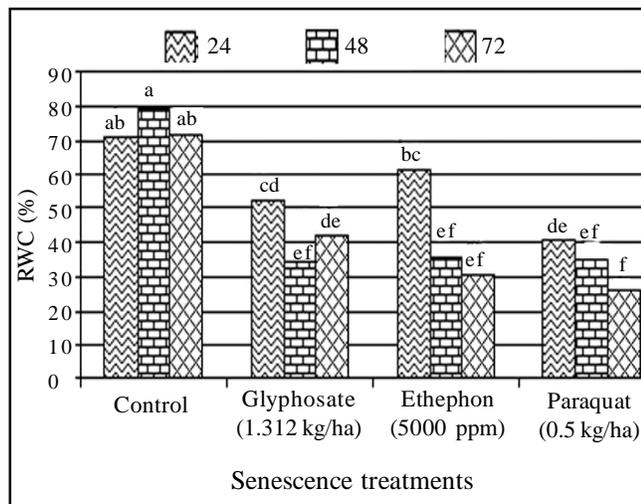


Fig. 4. Effect of glyphosate, paraquat and ethephon on membrane integrity in *Cyperus rotundus*

Membrane damage was much higher in case of paraquat and glyphosate than ethephon and control (Fig. 4). Initial membrane damage 24 h was more in glyphosate than paraquat, but later at 72 h significantly less damage compared to paraquat. Thus glyphosate induced senescence was better than paraquat but both were inferior to ethephon in membrane damage. Membrane intactness played an important role in efflux of herbicide into phloem depends on plasma membrane transporter (phPT1). Phosphate transporters are up-regulated during leaf senescence.

Leaf RWC in control treatment remained same and superior to herbicides and ethylene. Leaf RWC of glyphosate was on par with ethephon at all stages of senescence but had higher RWA than paraquat especially at 72 h glyphosate (Fig. 5). Thus glyphosate maintained RWC on par with ethephon which helped in enhanced phloem loading, translocation at 48 h after glyphosate application.

Biomass reduction over control was 96.5, 90.0 and 88% in 1.312+1.312, 3.28 and 1.312 kg/ha, respectively (Table 1). These data suggest that senescence induced by glyphosate at 48 h, *fb* glyphosate application enhanced the glyphosate efficacy. Exposure to ethylen eaccelerates leaf senescence whereas BA delays senescence (Grbic and Bleecker 1995). Total biomass reduction was assessed with anti-senescence BA (10  $\mu$ m) pre-treatment 48 h, *fb* glyphosate application. BA treatment reduced glyphosate efficacy by nine per cent compared to glyphosate (1.312 kg/ha). Thus, glyphosate induced leaves senescence at 48 h contribute 9% more to glyphosate efficacy by enhanced translocation owing to phosphate transporters are up-regulated during leaf senescence. During fall season (Novem-



**Fig. 5. Effect of glyphosate, ethephon and paraquat on relative water content in *Cyperus rotundus***

**Table 1. Effect of herbicide induced senescence on biomass of *C. rotundus***

Treatment	Total biomass (g)
Control	111.0 <sup>a</sup>
Glyphosate 3.28 kg/ha	10.9 <sup>cd</sup>
Glyphosate 1.312 kg/ha	13.2 <sup>c</sup>
Glyphosate 1.312 kg/ha + after 48 h 1.312 kg/ha	3.9 <sup>d</sup>
BA 10 μm + glyphosate 1.312 kg/ha	22.7 <sup>b</sup>
LSD (P=0.05)	8.5

ber to February), glyphosate had lowest ED<sub>75</sub> for control of *C. rotundus* (142 g/ha) than other growing seasons (329-1699 g/ha) due to scavenging of nutrients from senescent foliage (Devendra *et al.* 2005). Translocation of <sup>14</sup>C-glyphosate out of fed leaf was 10 and 5.8% during February and June, respectively (Chandranaik *et al.* 2004). Glyphosate (1.3 kg/ha) induced senescence maintained membrane integrity and high RWC than paraquat, which helped in enhanced phloem loading, translocation of glyphosate.

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