

Effects of glufosinate-ammonium and terbuthylazine on germination and growth of two weed species

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Summary

Effects of glufosinate-ammonium and terbuthylazine on germination and growth of two weed species were evaluated in the laboratory and in the greenhouse. Germination of *Asystasia gangetica* (L.) T. Anders. and *Paspalum conjugatum* Berg. declined considerably when seeds were germinated in petri dishes containing either glufosinate-ammonium or terbuthylazine at 250 mg L⁻¹.

Glufosinate-ammonium also caused a marked reduction in radicle elongation of *Asystasia*, whose fresh weight was reduced proportionately with increasing concentrations of either glufosinate-ammonium or terbuthylazine. Terbuthylazine was more effective than glufosinate-ammonium in reducing germination and growth of both weeds when applied either to seeds on the soil surface or to the soil prior to sowing. Storage of seeds after treatment with either herbicide reduced the inhibitory effect of the herbicide on germination and growth of the weeds. Residual terbuthylazine in soil 14 days after treatment could still reduce germination and growth of the weeds.

Introduction

Asystasia gangetica and *Paspalum conjugatum* are two weeds common in cocoa, rubber and oil palm plantations. A mature *A. gangetica* plant produces several fruits which contain four seeds each. The ripe fruits usually burst with considerable force, scattering the seeds one to three metres away from the mother plants (Lee 1981). *P. conjugatum* spreads quickly by means of its stolons as well as by seeds. Approximately 1500 seeds are produced per plant (Dirven 1971).

Such production of seeds contributes heavily to effective weed dispersal and establishment in plantations. Most agricultural soils contain a large reservoir of weed seeds which germinate over a period of time. Weed seeds shed onto the soil surface may remain there or be incorporated into the profile by natural or artificial means (Pareja *et al.* 1985). Potential weed problems in most cultivated crops including rubber and oil palm depend at least in part upon the weed seed bank. Norris (1985) suggests that weed control should exceed 99.99% efficiency to keep the seed bank static and proposed that an ideal weed control measure should

control 100% of the weed seed populations. Clearly, a weed control measure has to severely reduce the weed seed population in the soil to be effective. Research on the effects of herbicide on seeds in soil may contribute to knowledge on the effective control of weed seed populations.

The effects of herbicidal treatment on seed reserves in the soil or on subsequent growth of weed seedlings form an important aspect of weed control. However, not many studies have reported on the effects of herbicide on seed germination; it seems that only paraquat, glyphosate, pronamide, protham and metribuzin (Young *et al.* 1984, Salazar and Appleby 1982) have been studied for such effects. Glyphosate or paraquat applied directly to seeds on the soil surface may reduce germination and/or seedling growth of several grass species (Salazar and Appleby 1982, Egley and Williams 1978, Klingman and Murray 1976).

Glufosinate-ammonium is used in plantation crops such as cocoa, oil palm and rubber as well as in vegetable crops and on non-crop land to control a broad spectrum of grasses, broadleaf weeds, sedges and ferns (Kuah *et al.* 1989, Rodrigo 1988, Kassebeer *et al.* 1983). It is rapidly adsorbed to soil particles, effectively preventing degradation of the herbicide, or degraded by soil microorganisms, releasing carbon dioxide (WSSA 1989).

Terbuthylazine, a broad-spectrum s-triazine herbicide, is presently being evaluated in plantation crops in Malaysia. With adequate rainfall or irrigation, terbuthylazine is leached to deeper soil layers and is taken up by deep-rooted weeds. Terbuthylazine may move deeper into the soil, not necessarily because of its greater mobility, but because it persists longer (Bowman 1989). In the soil it prevents the germination of seedlings for several months after application (WSSA 1989).

Past research evaluating the effect of glufosinate-ammonium and terbuthylazine on the germination seeds of *A. gangetica* and *P. conjugatum* is very limited; most reports have considered only the control aspects at post-emergence stages. Glufosinate-ammonium and terbuthylazine, which have different actions on weed growth, were chosen for

this study. The objective of this study was, therefore, to evaluate the effects of these two herbicides on germination and seedling growth of two weed species prevalent in plantation crops of Malaysia.

Materials and methods

Weed seeds

Seeds of a broadleaf weed, *A. gangetica*, and a grass species, *P. conjugatum*, were collected from a rubber estate near Sungai Buluh, Selangor. After air-drying, harvested seeds were kept under laboratory conditions in air-tight glass bottles until used.

Herbicides

Glufosinate-ammonium (ammonium-DL-homoalanin-4-yl(methyl) phosphinate) was obtained from Hoechst (Malaysia) under the trade name Basta®; it contains 150 g a.i. L⁻¹. Terbuthylazine (2 tert-butylamino-4-chloro-6-ethylamino-5-triazine) was supplied by Ciba-Geigy (Malaysia) under the trade name Gardoprim®; it contains 500 g a.i. L⁻¹.

Laboratory experiment

Fifty seeds each of the two weed species were placed on 9 cm filter paper in a series of petri dishes that contained 8.0 mL of the herbicide solution or distilled water. The experimental design was a randomized complete block with five replications per treatment. The concentrations of glufosinate-ammonium or terbuthylazine used were 0 (control), 25, 50, 100 and 250 mg L⁻¹.

The petri dishes were placed in dark germination chambers at a constant temperature of 27°C. On days 3 through 10 daily germination counts were made. Seeds were considered germinated when the radicle had protruded through the seedcoat. Radicle length and fresh weight of 10 seedlings were recorded after 10 days. The experiment was repeated twice and the means were averaged. All data were subjected to an analysis of variance with mean separation by Duncan's multiple range test at 5% probability level.

Greenhouse experiments

Four experiments were conducted in the greenhouse to determine the effect of glufosinate-ammonium and terbuthylazine on seed germination and seedling growth of the two weed species. The herbicide was sprayed using a laboratory sprayer that delivered a volume of 500 L ha⁻¹ at a pressure of 200 kPa. The dose applied was 0 (control), 0.5 and 1.0 kg ha⁻¹. The soil used in the experiments was a sandy loam (40% sand, 40% silt and 20% clay) with an organic matter content of 1.1% and a pH of 6.2. No artificial light was supplied and the temperature in the greenhouse was 27±3°C.

Table 1. Germination, radicle length and fresh weight (% of control) of petri dish grown *Asystasia* and *Paspalum* 10 days after exposure to herbicides.

Treatment (mg L ⁻¹)	Germination		Fresh weight		Radicle length	
	<i>Asystasia</i>	<i>Paspalum</i>	<i>Asystasia</i>	<i>Paspalum</i>	<i>Asystasia</i>	<i>Paspalum</i>
Control	100 a	100 a	100 a	100 a	100 a	100 a
Glufosinate-ammonium						
25	72 b	49 d	48 b	65 bcd	5 c	60 b
50	68 b	15 e	41 bc	55 cd	4 c	32 d
100	36 c	7 ef	33 c	30 e	3 c	12 e
250	11 d	1 f	20 d	5 f	1 d	3 f
Terbuthylazine						
25	95 a	100 a	100 a	75 b	84 a	52 bc
50	17 d	97 ab	95 a	70 bc	84 a	44 bc
100	15 d	93 b	41 bc	55 cd	84 a	40 cd
250	6 d	83 c	20 d	55 cd	52 b	52 bc

Means within a column followed by the same letters are not significantly different at the 5% level by Duncan's multiple range test.

The four experiments (methods of application) were as follows:

- Herbicide applied directly to seeds. Fifty seeds of each species were placed in separate petri dishes, sprayed with the appropriate herbicide solution and dried at 20°C for three hours. The seeds were then placed firmly on an untreated, moist soil surface in individual plastic trays (30 cm × 30 cm × 10 cm).
- Herbicide applied to seed and soil. Seeds of each species were placed on the surface of moist soil in similar trays and then sprayed with the appropriate concentration of either glufosinate-ammonium or terbuthylazine.
- Storage of herbicide-treated seeds. Fifty seeds of each species were placed in separate petri dishes, sprayed with the appropriate herbicide treatment and dried at 27°C for 14 days. The seeds were then placed on the moist surface of untreated soil.
- Herbicides applied to soil. Soil in trays was pre-moistened to field capacity and then sprayed with the appropriate treatment. Fifty untreated seeds of each species were placed on soil surface on day 0 (immediately after treatment) and on day 14.

All plastic trays were placed in large metal trays in the greenhouse for four weeks and sub-irrigated as needed. Experimental design was a randomized complete block with five replications. After this growth period, percent of germination per tray and shoot length of five randomly sampled plants per tray were recorded. Shoots were then harvested, oven-dried at 50°C for three days and weighed. The experiment was conducted twice, and the means were averaged. All the data were subjected to analysis of variance followed by Duncan's multiple range test at 5% probability level.

Results and discussion

Laboratory experiment

After 10 days, each herbicide reduced germination of *Asystasia* proportionately with increasing herbicide concentration (Table 1). Seed germination of *Paspalum* declined significantly in the presence of 25 mg L⁻¹ glufosinate-ammonium and prevented seed germination at 250 mg L⁻¹. Terbuthylazine at 250 mg L⁻¹ caused a slight reduction in the germination of *Paspalum*. In the case of *Asystasia*, terbuthylazine at 50 mg L⁻¹ caused a greater reduction in seed germination (reduced by 83% of control) than did glufosinate-ammonium at the same concentration.

Fresh weight of *Asystasia* decreased proportionately with increasing concentrations of either herbicide. For instance, glufosinate-ammonium and terbuthylazine reduced fresh weight of *Asystasia* to 48% at 25 mg L⁻¹ and 41% of control at 100 mg L⁻¹, respectively. Fresh weight of *Paspalum* declined progressively in the presence of increasing concentrations of glufosinate-ammonium. Similarly, terbuthylazine did reduce the fresh weight of *Paspalum* but not by as much as did glufosinate-ammonium.

The petri dish experiment shows that glufosinate-ammonium reduced radicle elongation of the two species more than terbuthylazine. Radicle elongation of *Asystasia* was greatly reduced (95% of control) when germinated in glufosinate-ammonium at its lowest experimental concentration of a 25 mg L⁻¹. In contrast, terbuthylazine reduced radicle elongation of *Asystasia* significantly to 52% of control only at 250 mg L⁻¹, its highest experimental concentration. Radicle length of *Paspalum* was reduced proportionately with increasing glufosinate-ammonium concentration. On the other hand, increasing concentration of terbuthylazine had inconsistent effects on radicle length of the species.

The results of this experiment indicate that at higher concentrations, glufosinate-ammonium caused drastic reduction of radicle elongation of both species, but especially of *Asystasia*. Glufosinate-ammonium not only reduced germination of *Paspalum* and *Asystasia* at higher concentrations, it also consequently reduced fresh weight and radicle elongation. At concentrations as low as 50 mg L⁻¹, terbuthylazine caused a marked reduction of germination of *Asystasia*.

Greenhouse experiments

i. Herbicide applied directly to seeds

Direct application of glufosinate-ammonium to *Asystasia* seeds reduced germination more than did similar treatment with terbuthylazine. Treatment of *Asystasia* seeds with 1.0 kg ha⁻¹ glufosinate-ammonium caused 95% reduction in germination, compared to 85% when terbuthylazine was used (Table 2). Both herbicides also significantly reduced the germination of *Paspalum* but not as severely as they reduced germination of *Asystasia*.

Both glufosinate-ammonium and terbuthylazine significantly reduced the shoot dry weight and shoot length of *Asystasia* compared to those of the control (Tables 3 and 4). Terbuthylazine caused greater reduction in shoot dry weight of *Asystasia* than did glufosinate-ammonium, but no significant differences were observed between the 0.5 and 1.0 kg ha⁻¹ rates for either herbicide. Shoot dry weight of *Paspalum* was also reduced when seeds were treated with either herbicide. Shoot length of *Paspalum* was greatly reduced when seeds were treated with either herbicide at 1 kg ha⁻¹.

The results of this experiment indicate that direct application of herbicides to seeds may cause considerable reduction in germination. Consequently, numbers of viable seeds may decline, thereby reducing seed populations in soil.

ii. Herbicide applied to seeds and soil

When glufosinate-ammonium was applied to exposed seeds on the soil surface, it was not as effective in reducing seed germination of either species as when applied directly to the seeds in petri dishes (Table 2). However, at the highest rate,

terbuthylazine caused a marked decrease in germination of *Asystasia* and *Paspalum* germination by 94 and 93%, respectively.

Shoot length of *Asystasia* was also affected more by terbuthylazine than by glufosinate-ammonium (Table 4). A marked reduction of shoot elongation of

Asystasia was observed at 0.5 kg ha⁻¹ of terbuthylazine with 88% reduction, while shoot elongation of *Paspalum* was unaffected by the same treatment. Shoot elongation of *Paspalum* was, however, significantly affected when treated with terbuthylazine at 1.0 kg ha⁻¹.

Table 2. Germination (% of control) of *Asystasia gangetica* and *Paspalum conjugatum* seeds.

Herbicide kg ha ⁻¹		Spray seeds only	Spray seeds on soil	Spray seeds store 14 days before planting	Spray soil seed immediately after	Spray soil seed 14 days after
<i>Asystasia gangetica</i>						
Untreated		100 a	100 a	100 a	100 a	100 a
Glufosinate	0.5	35 c	87 b	51 d	85 b	85 b
	1.0	5 e	69 c	40 e	72 c	71 c
Terbuthylazine	0.5	60 b	25 d	83 b	7 d	53 d
	1.0	15 d	6 e	73 c	2 d	15 e
<i>Paspalum conjugatum</i>						
Untreated		100 a	100 a	100 a	100 a	100 a
Glufosinate	0.5	77 c	82 b	84 b	58 b	89 b
	1.0	67 d	88 b	73 c	32 c	85 b
Terbuthylazine	0.5	88 b	17 c	91 ab	10 d	78 c
	1.0	65 d	7 d	91 ab	2 d	54 d

Means for each species within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 3. Dry shoot weight (% of control) of *Asystasia gangetica* and *Paspalum conjugatum* seedlings.

Herbicide kg ha ⁻¹		Spray seeds only	Spray seeds on soil	Spray seeds store 14 days before planting	Spray soil seed immediately after	Spray soil seed 14 days after
<i>Asystasia gangetica</i>						
Untreated		100 a	100 a	100 a	100 a	100 a
Glufosinate	0.5	48 b	44 b	15 c	81 b	84 b
	1.0	40 b	37 c	9 c	65 c	69 c
Terbuthylazine	0.5	29 c	4 d	49 b	23 d	54 d
	1.0	27 c	2 e	47 b	16 e	25 e
<i>Paspalum conjugatum</i>						
Untreated		100 a	100 a	100 a	100 a	100 a
Glufosinate	0.5	68 b	70 b	73 b	90 a	84 a
	1.0	39 c	46 c	56 bc	93 a	77 a
Terbuthylazine	0.5	59 b	33 c	65 b	100 a	41 b
	1.0	72 b	12 d	38 c	7 b	20 c

Means for each species within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 4. Shoot length (% of control) of *Asystasia gangetica* and *Paspalum conjugatum* seedlings.

Herbicide kg ha ⁻¹		Spray seeds only	Spray seeds on soil	Spray seeds store 14 days before planting	Spray soil seed immediately after	Spray soil seed 14 days after
<i>Asystasia gangetica</i>						
Untreated		100 a	100 a	100 a	100 a	100 a
Glufosinate	0.5	70 b	80 b	55 c	81 b	94 a
	1.0	59 b	70 c	41 c	72 b	97 a
Terbuthylazine	0.5	54 b	12 d	78 b	59 c	97 a
	1.0	67 b	6 e	78 b	22 d	66 b
<i>Paspalum conjugatum</i>						
Untreated		100 a	100 a	100 a	100 a	100 a
Glufosinate	0.5	50 c	75 b	75 b	75 ab	93 ab
	1.0	56 bc	38 c	68 bc	50 bc	93 ab
Terbuthylazine	0.5	75 b	100 a	57 c	87 ab	73 b
	1.0	43 c	13 c	39 d	25 c	27 c

Means for each species within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

The reduction in shoot dry weight of the seedlings seems to follow naturally after decreased germination and shoot elongation; all these are effects attributable to herbicide injury. Growth of *Asystasia* was more sensitive to treatment with terbuthylazine. Shoot dry weight of *Asystasia* and *Paspalum* decreased by 98 and 88% of control, respectively, when terbuthylazine was applied at 1 kg ha⁻¹ (Table 3). This result shows that terbuthylazine applied to seeds on the soil surface was effective in reducing shoot dry weight of both species. The relative ineffectiveness of glufosinate-ammonium was probably due to its deactivation by soil adsorption. Earlier reports have shown that other non-residual herbicides such as paraquat and glyphosate did not affect shoot length and shoot dry weight of the grass species (Young *et al.* 1984) because these herbicides become inactive upon contact with soil particles (Knight and Tomlinson 1967). It should be noted that the activity of certain herbicides in soil is greatly affected by such factors as soil type, soil moisture and soil organic content (Knight and Tomlinson 1967). Increasing amounts of organic matter may reduce phytotoxicity effects of herbicides; perhaps this is the case with glufosinate-ammonium. Terbuthylazine is a soil-applied herbicide, therefore its activity in soil is higher than that of glufosinate-ammonium. The results of this experiment imply that under field conditions, application of terbuthylazine as pre-emergence herbicide might reduce germination and growth of certain weed species such as *Asystasia*.

iii. Effect of seed storage on germination and growth

Storage of glufosinate-ammonium treated seeds for two weeks before planting tended to have results similar to those of the experiment where seeds were sown immediately after treatment. However, the percentage of germination was in general higher than when seeds were not stored. Terbuthylazine did not have much effect on germination of *Paspalum* (Table 2). Other experiments have shown that storing seed of *Asystasia* for two weeks after collection could increase their germination rate (Ismail *et al.* 1990). The increase in the germination rate after storing may be due either to the greater seed maturity or to the possible failure of herbicides to penetrate the seeds during the storage period.

Shoot dry weight of *Asystasia* showed significant reduction when seeds were treated with glufosinate-ammonium (Table 3). Shoot dry weight of *Paspalum* was also reduced when treated with either glufosinate-ammonium or terbuthylazine. Shoot length of *Paspalum* was greatly reduced when treated with terbuthylazine at the higher concentration,

whereas *Asystasia* shoot length was similarly reduced by treatment with glufosinate-ammonium at the higher concentration (Table 4).

iv. Herbicides applied to soil.

When applied to soil, glufosinate-ammonium was not as effective in reducing seed germination of *Asystasia* as when applied directly to seeds (Table 2). Even at the higher level, glufosinate-ammonium significantly reduced shoot dry weight only of *Asystasia*. Interestingly, while terbuthylazine drastically reduced germination of *Paspalum* at both lower and higher rates, it significantly affected shoot dry weight and shoot length at only the higher rate.

Germination, shoot dry weight and shoot length of *Paspalum* were not affected as much when glufosinate-ammonium-treated soil was stored for 14 days before planting of seeds (Tables 2, 3 and 4). The results of this experiment on *Asystasia* show virtually no differences whether glufosinate-ammonium-treated soil was stored or not, except for a considerable increase in shoot length when treated soil had been stored. No significant difference was observed in germination, shoot dry weight and shoot length of *Paspalum* between the two concentrations of glufosinate-ammonium when treated soil had been stored before planting of seeds. In contrast, germination and shoot dry weight of *Asystasia* declined significantly with increasing concentration of glufosinate-ammonium applied to soil and kept for 14 days before sowing.

In general, incubation of terbuthylazine treated soil for 14 days before planting reduced the effect of the herbicide on the germination and growth of both species. However, the germination rates and growth of these species decreased with increasing terbuthylazine concentrations (Table 2). Again, seeds of *Asystasia* appeared very sensitive to terbuthylazine, especially at the higher rate. This study shows that herbicidal activity in soil, especially of glufosinate-ammonium, had declined during the 14-day incubation period. This result lead us to suspect that the rates of herbicide degradation or bioactivity differ between the two compounds. The degradation of glufosinate-ammonium in soil is rapid due to soil microbial activity (WSSA 1989). Field studies demonstrate that under natural conditions there is no translocation of glufosinate-ammonium into soil layers deeper than 15 cm (Behrendt *et al.* 1990). This clearly indicates the rapid biodegradation of the active ingredient (Bowman 1989). This compound is rapidly decomposed to 3 methyl phosphinicopropionic acid and finally to carbon dioxide (Behrendt *et al.* 1990). However, we found that, in the first two weeks after application terbuthylazine

lost its effectiveness faster in soil than glufosinate-ammonium. It has been reported that the activity of s-triazine herbicides remained almost constant for several months under glasshouse conditions (Horowitz 1969). An initial lag period of slow degradation has been observed with various herbicides, attributed to the adaptation of the microorganisms to the new compound (McCormick and Hiltbolt 1966). Soil microbes may take a longer time to recover in soil treated with terbuthylazine. Therefore its residual activity could be detected especially when applied at higher than the recommended rate (0.4 kg ha⁻¹). Evidence for this is the lower rate of *Asystasia* germination in the presence of terbuthylazine even 14 days after treatment.

As mentioned earlier, both weed species are serious weed problems in rubber, oil palm and vegetable crops. Their seeds have the capability to germinate and establish themselves while on the soil surface. Results of this study suggest that these species can be controlled to some extent by terbuthylazine prior to germination. Application of terbuthylazine generally reduced seed germination and/or seedling growth, but this effect was dependent upon weed species and seed-herbicide contact. Further experiments on persistence of terbuthylazine under field conditions need to be carried out, especially under tropical conditions and over a range of soil types.

Acknowledgements.

This work was supported by research grant IRPA 01-07-03-011. The authors express their sincere thanks to Dr. Anis Rahman from Ruakura Agricultural Centre, New Zealand for commenting on the manuscript. Thanks are also due to Ciba-Geigy Malaysia for providing terbuthylazine.

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