Research reports

Antagonism of glufosinate ammonium activity caused by glyphosate in the tank mixtures used for control of goosegrass (*Eleusine indica* Gaertn.)

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Abstract
Tank-mix combination experiments were conducted under glasshouse conditions to study the efficacy of tank mixtures of glyphosate plus glufosinate ammonium for the control of paraquat-resistant (R) and susceptible (S) biotypes of goosegrass at the young (3 to 4 leaves) and mature (2 to 4 tillers) stages. Fresh weight reduction for all mixtures of glyphosate and glufosinate ammonium were less (P <0.05) than the predicted value of fresh weight reduction, indicating strong antagonism between the two herbicides regardless of the growth stage. Although all tank mixtures of glyphosate plus glufosinate ammonium showed antagonistic reactions to each other with regard to their effect on the growth of goosegrass, these combinations still offered moderate to good control of both biotypes ranging from 68 to 89%. Comparison on the cost of application of tank mixtures revealed that the effective and economic rate of the herbicide combination treatment was a mixture of glyphosate at 75 g a.i. ha^{-1} with glufosinate ammonium at 25 g a.i. ha^{-1} at the 3 to 4-leaf stage. At the 2 to 4-tiller stage, it was found that 150 g a.i. ha^{-1} glyphosate plus 50 g a.i. ha^{-1} glufosinate ammonium was the most cost-effective combination compared to the other combinations. These tank mixtures reduced fresh weight of both biotypes by 86% at both growth stages.

Keywords: Joint action, goosegrass, antagonism, glyphosate, glufosinate ammonium.

Introduction
Herbicides have become increasingly important in agriculture and this reliance has resulted in an increase in the number of herbicide-resistant weed populations. Herbicide resistance is likely to occur when herbicides that have same site of action are used repeatedly in the same growing season or on the same area year after year (Gressel 1985).

Three herbicides namely paraquat, glyphosate and glufosinate ammonium have been widely used to control goosegrass (*Eleusine indica* (L.) Gaertn.) in Malaysia. However, due to frequent application of these herbicides, goosegrass populations have developed resistance towards paraquat (Itoh *et al.* 1990), glyphosate (Lim and Ngim 2000) and fluazifop-butyl (Marshall *et al.* 1993). This has caused problems in weed control because goosegrass is a common noxious weed infesting young oil palm and rubber plantations as well as vegetable farms and orchards due to its high fecundity and broad spectrum of tolerance to various environmental factors (Holm *et al.* 1977).

Glyphosate is a broad-spectrum herbicide used in crop and non-crop weed control. It is non-selective and very effective on deep-rooted perennial species, annual and biennial species of grasses, sedges and broadleaf weeds. Glufosinate ammonium is a non-selective water soluble herbicide. It controls both annual and perennial grasses as well as broadleaf weeds (Humburg *et al.* 1989). Glyphosate and glufosinate ammonium act by inhibiting 5-enoyl-pyruvyl shikimic acid 3-phosphate synthase and glutamine synthase, respectively (Humburg *et al.* 1989).

Tank-mix combinations have often been preferred over sequential applications because it saves time, costs less than applying each herbicide individually, and usually increases the spectrum of weed control. The efficacy of a tank-mix combination may be predicted from the control achieved when each herbicide is applied individually. Frequently, however, the control achieved by a tank-mix combination differs from the predicted control. The joint action of herbicides in combination is described as 'antagonistic' if the actual control is less than the predicted control and 'synergistic' if the actual control is greater than the predicted control and 'additive' if the weed control from the tank-mix combination is equivalent to the predicted control (Barrett 1993).

Many studies have been done on the control of goosegrass with a combination of two herbicides (Hydrick and Shaw 1994, Johnson 1996, Nishimoto and Murdoch 1999). Starke and Oliver (1998) studied the joint action of glyphosate with chlorimuron, fomesafen, imazethapyr and sulfentrazone at the 3 to 4-leaf stage. All the four fomesafen plus glyphosate combinations were found to have antagonistic effects on each other in controlling goosegrass. Glyphosate plus sulfentrazone tank mixtures were antagonistic at three of the four combinations for goosegrass, indicating that these herbicides were not complementary in tank mixtures. Chuah *et al.* (2004) demonstrated that tank mixing 1.44 or 2.88 kg a.i. ha^{-1} glyphosate with 0.60 or 1.20 kg a.i. ha^{-1} sethoxydim resulted in an antagonistic response on the glyphosate-resistant biotype at the 2 to 4-tiller stage.

Previous studies have shown that sethoxydim at 1.20 kg a.i. ha^{-1} is effective in controlling the glyphosate-resistant biotypes (Chuah *et al.* 2004). However, to date, there are no published data on the effect of glyphosate plus glufosinate ammonium on paraquat-resistant (R) and susceptible (S) biotypes of goosegrass. Tank combinations of glufosinate ammonium and glyphosate with different modes of action from paraquat were examined to reveal the potential of these mixtures as a replacement for paraquat. The objective of this study was: 1) to determine the efficacy of tank-mix combinations of glyphosate and glufosinate ammonium on the control of the R and S biotypes of goosegrass and 2) to evaluate the potential of antagonistic, synergistic or additive joint action of herbicide combinations applied for the control of the R and S biotypes.

Materials and methods
Seed collection
Goosegrass (*Eleusine indica*) seeds were collected from an oil palm plantation of the Rubber Industry Smallholders'

Development Authority (RISDA) at Gerdong, Setiu, Terengganu. Mature seeds of the paraquat-resistant (R) biotype were collected from a young oil palm plantation with a history of repeated use of paraquat since 1975 where control with this herbicide had failed. The paraquat-susceptible biotype (S) seeds were collected along the roadside near the young oil palm plantation, which had no history of paraquat use. A preliminary study on dose-response tests was conducted, using the 3 to 4-leaf stage plants of the R and S biotypes under glasshouse conditions (Teh 2005). The results showed that the R/S ratio of ED_{50} (paraquat dose that caused a 50% reduction in shoot fresh weight) was approximately two-fold.

Herbicides

The herbicides used in the study were glyphosate isopropylamine salt (Roundup Transorb®) containing 410 g a.i. L^{-1} solution and glufosinate ammonium (Basta 15®) containing 135 g a.i. L^{-1} solution.

Herbicide combination experiments

Seeds of the R and S biotypes were germinated at the soil surface in 28 cm × 36 cm × 5 cm trays containing commercial potting soil (Vriezenveen®) in the glasshouse. After one week, uniform sized seedlings were transplanted into 12 cm diameter polybags containing clay soil (clay 76.5%, silt 12.8%, sand 10.7%, pH = 5.6). These plants were watered twice daily and fertilized with one-gram organic fertilizer once per week. The plants were treated with a single herbicide, namely glyphosate or glufosinate ammonium or a combination of glyphosate plus glufosinate ammonium at two weeks (3 to 4 leaves) and five weeks (2 to 4 tillers) after germination using a compression sprayer (Matabi Style 7) with a flat-fan nozzle, calibrated to deliver 450 L ha^{-1} at 200 kPa.

The experimental design was a randomized complete block with a factorial arrangement of the treatments with three replications. Each treatment had four plants. Glyphosate was applied at the rates of 75, 150 or 300 g a.i. ha^{-1} while glufosinate ammonium was applied at the rates of 6.25, 12.5 or 25 g a.i. ha^{-1} both with and without glyphosate for young plants (3 to 4 leaves). For mature plants (2 to 4 tillers), glyphosate was applied at the rates of 150, 300 or 600 g a.i. ha^{-1} while glufosinate ammonium was applied at the rates of 12.5, 25 or 50 g a.i. ha^{-1} both with and without glyphosate for immature plants. The ratio of glyphosate to glufosinate ammonium in the mixtures varied from 48:1 to 3:1. Based on the herbicide labels, the recommended rate for glyphosate and glufosinate ammonium was 1230 g a.i. ha^{-1} and 450 g a.i. ha^{-1}, respectively.

The above ground fresh weight samples were measured at two and three weeks after treatment for young and mature plants, respectively. The fresh weight reduction was calculated as:

$$100 - ((\text{plant fresh weight} / \text{untreated plant fresh weight}) \times 100) \quad \text{(Eq. 1)}$$

Arcsine square root transformation was performed on the fresh weight reduction data before analysis. The non-transformed data were statistically interpreted based upon the transformed data. The transformed fresh weight reduction data were subjected to analysis of variance (ANOVA) and the multiplicative survival model (Colby, 1967). The fresh weight reduction data were compared using Tukey's Honestly Significant Difference (HSD) test at the 5% level of significance. A comparison was carried out on the cost of each tank mixture. The equation used for calculating the expected response was as follows:

$$E = 100 - ((100 - x) \times (100 - y) / 100) \quad \text{(Eq. 2)}$$

where E = expected growth reduction as a percentage of the control, x = growth reduction as percentage control from glyphosate, y = growth reduction as percentage control from glufosinate ammonium.

Results and discussion

Table 1 shows the effect of the tank-mix combination of glyphosate and glufosinate ammonium on the control of both biotypes at the 3 to 4-leaf stage. The R and S biotypes of goosegrass showed no significant difference ($P > 0.05$) in response to a combination of glyphosate and glufosinate ammonium, implying that no negative cross resistance is observed. Therefore, data of both the R and S biotypes were combined for further analysis. Based on shoot fresh weight reduction, glyphosate provided poor control of both biotypes ranging from 34 to 60%, while glufosinate ammonium provided better control that ranged from 71 to 86% for both biotypes.

In total, nine different binary mixtures of glyphosate and glufosinate ammonium were studied. It was found that the estimated percentage reduction for the nine mixtures was significantly ($P < 0.05$) lower than the predicted percentage reduction, implying that all the mixtures were antagonistic towards the control of goosegrass (Table 1). Glufosinate ammonium applied alone at 6.25 g a.i. ha^{-1} provided 71% control of both biotypes. The addition of 75, 150 or 300 g a.i. ha^{-1} of glyphosate did not improve the control. However, when glufosinate ammonium at 12.5 g a.i. ha^{-1} was tank mixed with different doses of glyphosate at 150 g a.i. ha^{-1} or 300 g a.i. ha^{-1}, an increase in percentage fresh weight reduction was observed when compared to the application of glufosinate ammonium alone at 12.5 g a.i. ha^{-1}. Glufosinate ammonium at 25 g a.i. ha^{-1} reduced plant fresh weight by 86%. There was no significant difference in reduced shoot fresh weight after the addition 150 of or 300 g a.i. ha^{-1}

Table 1. Shoot fresh weight reduction of paraquat-resistant and susceptible biotypes of goosegrass at the 3 to 4 leaf stage in the glasshouse two weeks after application of the herbicide mixture.

Herbicide combination treatment (g a.i. ha^{-1}) Glyphosate + glufosinate ammonium	Fresh weight reduction (%)	Cost of herbicide (US\$ ha^{-1})
0 + 6.25	71 g[A]	0.34
0 + 12.5	78 e	0.68
0 + 25	86 bc	1.35
75 + 0	34 j	0.51
150 + 0	50 i	1.01
300 + 0	60 h	2.02
75 + 6.25	68 h (81)[B] –[C]	0.84
150 + 6.25	72 g (86) –	1.35
300 + 6.25	74 g (90) –	2.36
75 + 12.5	76 ef (86) –	1.18
150 + 12.5	83 d (89) –	1.69
300 + 12.5	85 cd (92) –	2.70
75 + 25	86 bc (91) –	1.86
150 + 25	88 ab (93) –	2.36
300 + 25	89 a (95) –	3.37

[A] Means followed by the same letter are not significantly different according to Tukey Honestly Significantly Different (HSD) at P = 0.05.
[B] Values in parentheses are the expected value as calculated by Colby's (1967) method.
[C] – denotes antagonism.

glyphosate compared to the control provided by glufosinate ammonium alone at 25 g a.i. ha^{-1} (Table 1).

The results of this study demonstrated that tank mixtures of 75, 150 or 300 g a.i. ha^{-1} glyphosate plus 25 g a.i. ha^{-1} glufosinate ammonium and a combination of 300 g a.i. ha^{-1} glyphosate plus 12.5 g a.i. ha^{-1} glufosinate ammonium gave good control of 85 to 89% for the R and S biotypes at the 3 to 4-leaf stage. Based on economic analysis, it was found that the tank-mix combination of glyphosate at 75 g a.i. ha^{-1} plus glufosinate ammonium at 25 g a.i. ha^{-1} is the most cost-effective among the combinations.

Table 2 shows the effect of tank-mix combinations of glyphosate and glufosinate ammonium on the control of both biotypes at the 2 to 4-tiller stage. Again the R and S biotypes of goosegrass showed no significant difference ($P > 0.05$) in response to combinations of glyphosate and glufosinate ammonium and data of both biotypes were combined for further analysis. Based on shoot fresh weight reduction, glyphosate provided poor control that ranged from 40 to 65% for both biotypes, while fresh weight reduction for both biotypes ranging from 74 to 86% was obtained when treatment was done with glufosinate ammonium.

It was found that the estimated percentage reduction was significantly ($P < 0.05$) lower than the predicted percentage reduction in all nine binary mixtures. This indicates that antagonism occurs in all tank mixtures of glyphosate plus glufosinate ammonium when used to treat both the R and S biotypes (Table 2). Glufosinate ammonium applied alone at 12.5 g a.i. ha^{-1} provided 74% control of both biotypes. However, the tank mixture of glufosinate ammonium at 12.5 g a.i. ha^{-1} with glyphosate of 150 g a.i. ha^{-1} acted antagonistically and reduced shoot fresh weight to 71%. When glufosinate ammonium at 25 g a.i. ha^{-1} was tank mixed with different doses of glyphosate at 300 or 600 g a.i. ha^{-1}, an increase in percentage fresh weight reduction was observed compared to fresh weight reduction caused by 25 g a.i. ha^{-1} glufosinate ammonium applied alone. However, there was no significant difference in reduced shoot fresh weight when 25 g a.i. ha^{-1} glufosinate ammonium was applied with 150 g a.i. ha^{-1} glyphosate, compared to glufosinate ammonium applied alone at 25 g a.i. ha^{-1}. Glufosinate ammonium applied alone at 50 g a.i. ha^{-1} was effective in reducing plant fresh weight by 86%. The addition of glyphosate at 600 g a.i. ha^{-1} increased plant fresh weight reduction of both biotypes to 89%.

The results of the present study showed that 150, 300 or 600 g a.i. ha^{-1} glyphosate plus 50 g a.i. ha^{-1} glufosinate ammonium as well as 600 g a.i. ha^{-1} glyphosate plus 25 g a.i. ha^{-1} glufosinate ammonium provided good control (≥85%) of both biotypes at the 2 to 4-tiller stage. Comparison of the cost of herbicides, however, revealed that a tank mixture of glyphosate at 300 g a.i. ha^{-1} with glufosinate ammonium at 25 g a.i. ha^{-1} was the most cost-effective combination.

Although application of glufosinate ammonium alone at 25 and 50 g a.i. ha^{-1} was effective in controlling both biotypes of goosegrass at the young and mature stages, respectively, it is not an ideal approach for controlling goosegrass because single herbicide application may only be effective for short-term weed control. The weed may become resistant to that particular herbicide after several years. Tank mixtures can be beneficial in weed control and they also delay the occurrence of resistance to both herbicides applied in a herbicide combination (Powles 1997). Both glyphosate and glufosinate ammonium have different modes of action and exhibit low persistence in the agroecosystem (Humburg *et al.* 1989). These are important criteria in selecting two herbicides for a mixture as suggested by Wrubel and Gressel (1994) to prevent or preclude resistance evolution.

All mixtures of glyphosate and glufosinate ammonium were less effective than the predicted value of fresh weight reduction irrespective of the growth stage, indicating strong antagonism between the two herbicides (Tables 1 and 2). This result is in agreement with a previous study that showed a mixture of both commercial glufosinate ammonium plus glyphosate at the ratio varying from 20:80 and 75:25 on weed species such as *Sinapis arvensis* L. (charlock) and *Sinapsis alba* L. were antagonistic (Kudsk and Mathiassen 2004). Likewise, both *S. arvensis* and *S. alba* were also found to exhibit antagonistic responses when treated with 16 combinations of glufosinate ammonium and analytical grade glyphosate (Kudsk and Mathiassen 2004).

The mechanism by which joint action was observed for glufosinate ammonium and glyphosate mixtures may be due to physiological antagonism. It is noted that glufosinate ammonium is a fast-acting contact herbicide whereas glyphosate is a slow-acting, systemic herbicide. Glufosinate ammonium may have destroyed the leaf tissue before glyphosate was translocated to others parts of the plant such as the roots and stem. Further studies are yet to be done to examine the uptake and translocation of tank mixtures of glyphosate plus glufosinate ammonium in both biotypes of goosegrass.

The results of this study show that combinations of glyphosate plus glufosinate ammonium can provide moderate to good control of both biotypes of goosegrass, although these mixtures acted antagonistically. Furthermore, rates of tank mixtures used in the study are greatly reduced from 16 to 72 times lower than the currently recommended rates for single herbicide application, thereby reducing the cost of weed control for farmers as well as

Table 2. Shoot fresh weight reduction of paraquat-resistant and susceptible biotypes of goosegrass at the 2 to 4 tiller stage in the glasshouse three weeks after application of the herbicide mixture.

Herbicide combination treatment (g a.i. ha^{-1}) Glyphosate + glufosinate ammonium	Fresh weight reduction (%)	Cost of herbicide (US$ ha^{-1})
0 + 12.5	74 g[A]	0.68
0 + 25	79 e	1.35
0 + 50	86 bc	2.70
150 + 0	40 k	1.01
300 + 0	52 j	2.02
600 + 0	65 i	4.04
150 + 12.5	71 h (85)[B] –[C]	1.69
300 + 12.5	75 g (88) –	2.70
600 + 12.5	76 fg (91) –	4.72
150 + 25	78 ef (87) –	2.36
300 + 25	84 d (90) –	3.37
600 + 25	85 cd (93) –	5.39
150 + 50	86 bc (91) –	3.71
300 + 50	88 ab (93) –	4.72
600 + 50	89 a (95) –	6.74

[A] Means followed by the same letter are not significantly different according to Tukey Honestly Significantly Different (HSD) at P = 0.05.
[B] Values in parentheses are the expected value as calculated by Colby's (1967) method.
[C] – denotes antagonism.

managers of plantations. However, glasshouse results cannot be directly extrapolated to field conditions since the field responses to post-emergence herbicides such as glyphosate and glufosinate ammonium are likely to be influenced by environmental conditions of soil moisture (Adkins *et al.* 1998), relative humidity (Ramsey *et al.* 2002) and light intensity (Ismail and Ibrahim 1996) before, at and after the herbicide application. Hence, further studies are yet to be undertaken in the field at a range of sites to verify the findings from the glasshouse experiments.

Acknowledgement

This research was supported by UMT fundamental research grant 54152.

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