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RESEARCH PAPER

Preliminary findings of potentially resistant goosegrass (*Eleusine indica*) to glufosinate-ammonium in Malaysia

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Goosegrass (*Eleusine indica*), regarded as one of the world's worst weeds, is highly pernicious to cash crop-growers in Malaysia. Following reports in 2009 that glufosinate-ammonium failed to adequately control goosegrass populations in Kesang, Malacca, and Jerantut, Pahang, Malaysia, on-site field trials were conducted to assess the efficacy of glufosinate-ammonium towards goosegrass in both places. Preliminary screenings with glufosinate-ammonium at the recommended rate of 495 g ai ha^{-1} provided 82% control of the weed at a vegetable farm in Kesang, while the same rate failed to control goosegrass at an oil palm nursery in Jerantut. The ensuing greenhouse evaluations indicated that the "Kesang" biotype exhibited twofold resistance, while the "Jerantut" biotype exhibited eightfold resistance towards glufosinate-ammonium, compared to susceptible goosegrass populations. The occurrence of glufosinate resistance in goosegrass calls for more integrated management of the weed to prevent escalating resistance and further proliferation of the weed in Malaysia.

Keywords: glufosinate-ammonium, goosegrass, herbicide resistance, integrated weed management.

Goosegrass (*Eleusine indica* [L.] Gaertn.) is listed as one of the world's worst weeds (Holm *et al*. 1977). In Malaysia, goosegrass, otherwise known locally as "rumput cakar ayam", is known for its obnoxious and competitive traits. The weed grows best in moist, derelict, otherwise fertile, cultivated soil in full sunlight and, once established, is difficult to eradicate (Swarbrick 1997). A single goosegrass plant is capable of producing ≤140 000 seeds. Being an annual and rhizomatous weed, goosegrass matures and propagates rapidly. Predominantly found in fruit-cultivation and vegetable farms, orchards, and young palm oil nurseries, goosegrass poses a threat to cash crops, causing a loss of food quality and quantity to farms and plantations (Mohd *et al*. 2008).

Herbicides provide reliable and economical control of goosegrass and other weeds to farmers in vegetable and fruit orchards in Malaysia. However, the heavy reliance on herbicides has shown some adverse effects. In 1989, the first case of fluazifop-butyl-resistant goosegrass in Malaysia, due to repetitive usage, was reported (Leach *et al*. 1993). The resistance of goosegrass towards glyphosate, one of the most extensively used herbicides in Malaysian agriculture, was reported in 1997 (Dill *et al*. 2000; Lee & Ngim 2000). The resistance of goosegrass biotypes towards acetyl coenzyme A carboxylase inhibitors and glycine herbicides has been reported in Malaysia since 1989 and 1997, respectively (Leach *et al*. 1993; Dill *et al*. 2000; Lee & Ngim 2000).

The development of herbicide resistance in weeds is an evolutionary process that occurs generally in response to the selection pressure that is imposed by the frequent use of one or more herbicides with the same mode of action (Powles *et al*. 1998; Lorraine-Colwill *et al*. 2003; Powles S. B., 2009, personal communication). As such, farmers have reverted to herbicides with different modes of action to control goosegrass.

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Glufosinate-ammonium is a broad-spectrum, non-selective, systemic herbicide that controls several annual and perennial grasses and broad-leaved weeds (Ahrens 1994). It was introduced into Malaysia in 1985 under the commercial name of "Basta®". Glufosinate works by inhibiting the activity of glutamine synthetase, the enzyme that converts glutamate plus ammonia to glutamine. The accumulation of ammonia in the plant destroys the plant cell. This causes photosynthesis to be severely inhibited. Ammonia reduces the pH gradient across the membrane, which can uncouple photophosphorylation. It was used in small quantities during the early years of its introduction. However, with the recent development of >100 varieties of glufosinate-resistant plants and the increasing resistance of weeds to glyphosate and other herbicides, glufosinate-ammonium usage is significantly increasing throughout the world and Malaysia.

In early 2009, we received reports from a farmer in Kesang, Malacca, and from planters at an oil palm nursery in Jerantut, Pahang, Malaysia, that glufosinate-ammonium failed to adequately control the goosegrass population at their respective place. In response, an investigation was conducted to examine both goosegrass populations (the "Kesang" and "Jerantut" biotypes) from 2008 to 2010 in order to ascertain that resistance to the herbicide did occur and to determine the level of resistance among those biotypes.

This article reports on the first case of glufosinate-ammonium resistance among goosegrass populations in Malaysia.

MATERIALS AND METHODS

A field trial was set up in the farmer's vegetable farm in Kesang and in the oil palm nursery in Jerantut. Plots of 2 m × 1 m were laid out, with three replicates for each plot, and were arranged accordingly in a randomized complete block design. Glufosinate-ammonium (Syngenta Crop Protection Sdn. Bhd., Shah Alam, Malaysia.) was sprayed onto *Eleusine indica* plants by using a sprayer with a flat-fan nozzle that was calibrated to deliver 450 L ha^{-1} (PB-20 knapsack sprayer; Cross Mark, Kluang, Malaysia.) at four different rates, ranging from 247.5 g ai ha^{-1} to 1980 g ai ha^{-1} (Kesang farm) and from 495 g ai ha^{-1} to 3960 g ai ha^{-1} (Jerantut oil palm nursery). Most of the goosegrass had matured and was at the seed-producing stage. The goosegrass had > 90% coverage and the interaction with other weed species, if any, would have been minimal. The interactions with other weed species were not taken into consideration in this study. Visual estimates of the percentage damage due to the herbicide treatment, based on the level of leaf and stem necrosis on a scale of 0–100% (0 = "no damage", 100 = "total control"), were carried out at weekly intervals for four consecutive weeks.

In order to rule out environmental factors (e.g. rain, humidity, and light) and agronomic factors (e.g. soil type, water stress, and soil pH) that could affect the efficacy of the herbicide on goosegrass, cuttings from the field that survived the herbicide treatment were collected and transplanted into pots in a greenhouse at the Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia. In order to evaluate the resistance level of both the Kesang and Jerantut biotypes, samples of goosegrass that was susceptible towards glufosinate-ammonium were collected from urban housing areas with no history of herbicide treatment.

The cuttings of goosegrass were transplanted into unsterilized potting soil in 10 cm^2 pots with 0.3 cm of the shoot buried (a maximum period of 7 days was allowed until the cuttings were transplanted). The pots were kept inside the greenhouse and watered twice daily from above by using a fine rose. After the leaves had regenerated to ~3 cm long, the pots were moved outside the greenhouse to allow maximum sun exposure. Once the leaves were ~7–20 cm long, the goosegrass plants were treated with glufosinate-ammonium at 495, 990, 1980, and 3960 g ai ha^{-1} by using similar spray application equipment to that described earlier at a spray volume of 450 L ha^{-1}. The sampling and assessment of the herbicide's efficacy were based on the Syngenta Quick-test method (Boutsalis 2001), with slight modifications.

Visual estimates of the percentage damage of goosegrass following the glufosinate-ammonium treatments were carried out in the same manner as in the on-site field trial. The percentage of control of goosegrass as a result of glufosinate-ammonium treatment was subjected to a probit analysis (Finney 1971) by using the statistical software package of SPSS (v. 17.0; SPSS, Kuala Lumpur, Malaysia.) in order to determine the LC_{50} (the amount of glufosinate-ammonium that is required for 50% control) values. The resistance indices for each biotype also were calculated.

The data from the field and greenhouse experiments were collated and subsequently subjected to an ANOVA. Prior to the ANOVA, the percentage of control data were transformed to log + 5-values. Then, the treatment means were subjected to Scheffe's tests in order to determine the significant differences between them, if any.

RESULTS

Glufosinate-ammonium provided very good control of the goosegrass populations at the Kesang farm. Even at a

Table 1. Percentage control of goosegrass by different rates of glufosinate-ammonium 14 days after treatment

Biotype	Rate (g ai ha^{-1})	% Control	
		Field trial	Greenhouse
Kesang	247.5	77 d	NA†
	495	82 dA	35 bB
	990	94 eA	72 dB
	1980	97 eA	100 eA
	3960	NA†	100 e
Jerantut	495	0 aA	3 aA
	990	45 bA	37 bA
	1980	65 cA	28 bB
	3960	85 dA	64 cB

Values followed by the same upper-case letters in a row and those followed by the same lower-case letters in a column are not significantly different at $P < 0.05$ (Scheffe's test). † NA, not applicable or not tested.

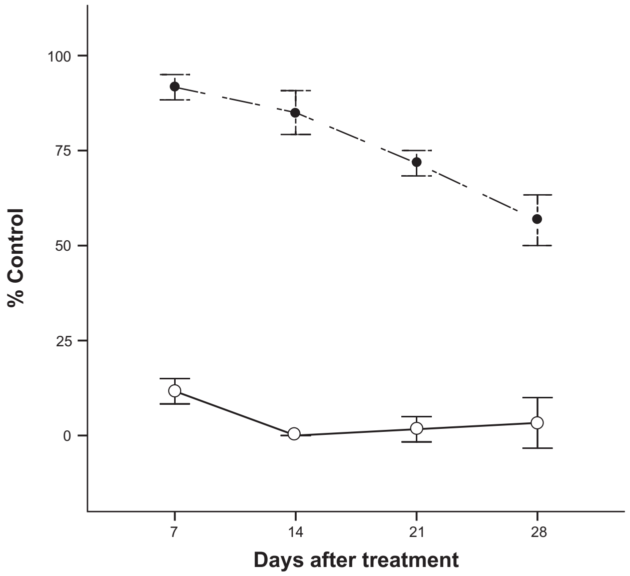

Fig. 1. Field evaluation of the differential response of the goosegrass biotype from Jerantut, Pahang, Malaysia, to glufosinate-ammonium at 495 g ai ha^{-1} (○) and 3960 g ai ha^{-1} (●). The bars represent 1 ± SD values.

sublethal dose of 247.5 g ai ha^{-1} (half of the recommended rate of application), 77% of the control was achieved 14 days after treatment (DAT). There were rate-mediated increases in the percentage control of goosegrass with glufosinate-ammonium. At fourfold the recommended rate (1980 g ai ha^{-1}), 97% control was achieved. However, the same level of efficacy of the herbicide did not manifest in the goosegrass populations in the greenhouse trial. At the recommended label rate of 495 g ai ha^{-1}, only 35% of the control was achieved. As the rate(s) increased, so did the level of control. A total annihilation (100% control) of the goosegrass populations for the Kesang biotype was achieved (100%) at fourfold and eightfold the recommended rate of application of the herbicide (Table 1).

Figure 1 illustrates the level of control of the goosegrass biotype from the Jerantut populations that were subjected to the recommended rate of 495 g ai ha^{-1} *vis-à-vis* 3960 g ai ha^{-1}, or eightfold the recommended rate of glufosinate-ammonium. Interestingly, at 495 g ai ha^{-1}, very poor control of the scourge was achieved, apparently with no sign of a breakdown in resistance with age. With 3960 g ai ha^{-1}, the herbicide showed measurable control against the weed, ranging from 65% to 85%. Intriguingly, there was a time-mediated reduction in the ability of the herbicide to kill the weed. These phenomena were exemplified by the initial 85% kill rate of the weed at 7 DAT, compared to an 82%, 77%, and 65% kill rate at 14, 21, and 28 DAT, respectively.

Glufosinate-ammonium, at the recommended application rate of 495 g ai ha^{-1}, failed to inflict any damage on the goosegrass populations in Jerantut (Table 1, Fig. 1). Nevertheless, the rate-mediated increase in the level of control of the goosegrass populations by the herbicide prevailed. For example, with 990 g ai ha^{-1}, 45% control was achieved and the percentage control increased by 20% with a twofold increase in the rate of glufosinate-ammonium that was used. In the greenhouse studies, glufosinate-ammonium produced a similar pattern of control against the Jerantut biotype, similar to those in the field trials. At 495 g ai ha^{-1}, only 3% of the control was achieved. The herbicide at 990, 1980, and 3960 g ai ha^{-1} produced 37%, 28%, and 64% control, respectively, against the Jerantut biotype of goosegrass at 14 DAT (Table 1).

While the treatment of 495 g ai ha^{-1} failed to cause any significant kill rate on the field populations of the Jerantut biotype of goosegrass, a similar treatment afforded a 38–48% kill rate on the Kesang biotype of goosegrass (Fig. 2). Despite measurable differences in the percentage kill of the scourge with time for both biotypes, such damage was not very significant.

The LC_{50} values, together with the Resistance Index values, for all three biotypes are shown in Table 2. The Kesang biotype had a Resistance Index value of 1.97 for glufosinate-ammonium. The parallel figure for the Jerantut biotype was 7.63.

DISCUSSION

Normally for marginal cases of resistance, a higher level of control of weeds is anticipated under greenhouse conditions as the recommended rate is more effective under these conditions (Heap 2005). This was not the case in our study, where a lesser percentage of control was achieved for both the Kesang and Jerantut biotypes of goosegrass. We believe that this is related to the selection process during sampling, where the goosegrass plants that survived the herbicide treatment in the field were collected for the greenhouse trial. It is possible that those cuttings exhibited a higher level of resistance towards glufosinate-ammonium.

The Syngenta Quick-test was adopted as it is robust, not dependent on seed availability, and is not influenced by seed dormancy (Boutsalis 2001). During the sampling, the goosegrass seeds were mostly not viable for the seed test as they were affected by the earlier herbicide treatment and/or by pests. The Syngenta Quick-test overcomes these problems as it involves using cuttings from the whole plant. It is also applicable to many other graminaceous and dicot weeds.

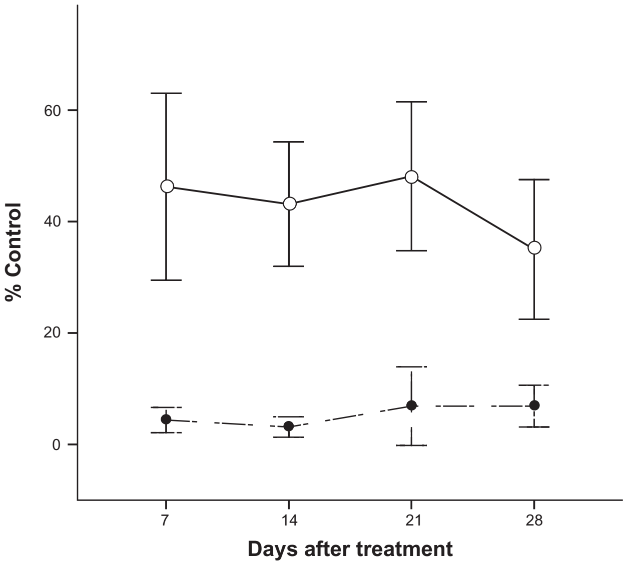

Fig. 2. Greenhouse evaluation of the differential response of the Kesang (○) and Jerantut (●) biotypes to the glufosinate-ammonium treatments at the recommended rate of 495 g ai ha^{-1}. The bars represent 1 ± SD values.

Theoretically, any Resistance Index value of >1 should be considered as showing resistance. However, Heap (2005) suggested that any Resistance Index value that is less than 10-fold should be considered as a low level of resistance or partial resistance. It is reasonable to believe that both the Kesang and Jerantut biotypes are developing resistance towards glufosinate, with the Jerantut biotype posing a more serious threat.

The treatment history revealed that the vegetable farmer in Kesang had started using glufosinate-ammonium only in the past 1.5 years after the previous glyphosate treatments failed to control the goosegrass population. In addition to the chemical control that the farmer had adopted, he ploughed his land each time before a new round of planting. In contrast, the planters in the oil palm nursery had relied solely on glufosinate-ammonium for weed control for the past 5 years, with a high intensity of spraying: there were as many as 24 spray rounds per annum. This high intensity of spraying might have led to a selection level, leaving only the resistant biotype remaining intact. Furthermore, the high fecundity of goosegrass, coupled with high selection pressure following repeated spraying with glufosinate-ammonium, might have resulted in more-resistant gooosegrass populations becoming dominant.

There is clear evidence that the Jerantut biotype is developing resistance to glufosinate (Figs 1 and 2, Table 2). The Kesang biotype, albeit having a resistance ratio of 1.97, still can be controlled with glufosinate-ammonium, but the ensuing repeated spraying might lead to the build-up of resistance to the herbicide among thriving populations. The control level of goosegrass by glufosinate-ammonium decreased gradually over time, a probable manifestation of the age-mediated breakdown of resistance among the treated populations or the reduced efficacy of the herbicide with time, perhaps related to the breakdown of the herbicide. Similar find-

Table 2. Amount of glufosinate-ammonium that is required for 50% control (LC_{50}) of the biotypes of goosegrass

Treatment	Biotype	LC_{50} (g ai ha^{-1})	Resistance Index value‡
Glufosinate-ammonium	Susceptible	301 (135–523)†	1.00
	Kesang	593 (347–903)	1.97
	Jerantut	2297 (1580–3594)	7.63

† Values in the parentheses represent the 95% confidence intervals; ‡ the Resistance Index is the ratio of the LC_{50} of the suspected resistant biotypes to that of the susceptible population. The values are LC_{50}-calculated by using a probit analysis on the data from the greenhouse experiments.

ings were recorded by others on age-mediated breakdown or the reduction in herbicide resistance by weeds (Baki 1980). The appearance of substantial resistance to glufosinate-ammonium in glufosinate-ammonium-selected field populations of goosegrass is truly worrying as this weed species previously has demonstrated resistance to other herbicides, such as fluazifop-butyl and glyphosate (Leach *et al*. 1993; Lee & Ngim 2000). That resistance now has appeared in glufosinate-ammonium-selected field populations of goosegrass. Previously, there have been reports in the UK and Japan of glufosinate-ammonium-resistant transgenes that have been transferred to the weedy relatives of experimental crops (Brown 2005; Saji *et al*. 2005). To our knowledge, there is no reported case of weeds that are resistant to glufosinate-ammonium due to selective pressure. Invariably, our data are indicative of being the first case(s) of proven or recorded resistance to glufosinate-ammonium among goosegrass populations in the world, in general, and in Malaysia, in particular. We advocate that integrated weed management should be adopted by those who are involved in agricultural practise in order to manage weed-resistance problems and to prevent the escalation of weed resistance to herbicides.

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