Malays. Appl. Biol. (2005) 34(2): 43–49

PARAQUAT AND GLYPHOSATE RESISTANCE IN WOODY BORRERIA (*HEDYOTIS VERTICILLATA*) GROWING AT OIL PALM PLANTATIONS IN TERENGGANU, MALAYSIA

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ABSTRACT

Glyphosate- and paraquat-resistant and susceptible biotypes of *Hedyotis verticillata* (woody borreria) infesting oil palm plantations of Terengganu, Malaysia, belonging to the Federal Land Consolidation and Rehabilitation Authority (FELCRA), Bukit Sudu, FELCRA Bukit Kapah, and the Federal Land Development Authority (FELDA), Belara, Kuala Terengganu were screened in a greenhouse study. Dose-response tests conducted using 3- to 4-leaf plants revealed that *H. verticillata* from oil palm plantations of FELCRA Bukit Kapah and FELDA Belara have developed multiple-resistance towards both glyphosate and paraquat. The paraquat resistance biotype of *H. verticillata* was detected in oil palm plantations of FELCRA Bukit Sudu. Comparison of concentrations of herbicides required to kill 50% (ED_{50} value) of the population indicated that the resistant biotypes of *H. verticillata* are between 2 to 4-fold more resistant than their respective susceptible biotypes.

ABSTRAK

Penyaringan ke atas biotip rintang dan rentan *Hedyotis verticillata* terhadap glifosat dan parakuat yang tumbuh di ladang kelapa sawit FELCRA Bukit Sudu, FELCRA Bukit Kapah dan FELDA Belara, Terengganu, Malaysia telah dijalankan di rumah kaca. Ujian gerak balas dos yang menggunakan tumbuhan pada peringkat 3-4 helai daun menunjukkan *H. verticillata* dari ladang kelapa sawit FELCRA Bukit Kapah dan FELDA Belara adalah rintang terhadap kedua-dua glifosat dan parakuat. Biotip *H. verticillata* yang rintang terhadap parakuat dikesan di ladang kelapa sawit FELCRA Bukit Sudu. Perbandingan antara kadar racun herba yang diperlukan untuk mematikan 50% daripada populasi (nilai ED_{50}) menunjukkan biotip rintang adalah di antara 2 – 4 kali ganda lebih rintang berbanding dengan biotip rentan masing-masing.

Key words: *Hedyotis verticillata*, multiple-herbicide resistance, ED_{50}, dose-response

INTRODUCTION

Herbicide resistance is global and not a new phenomenon. Resistance can occur when a particular herbicide is applied repeatedly for at least three to five years to sensitive species (Monaco *et al*., 2002). Resistance frequently occurs with herbicides that have very great efficacy to specific weed species. This is because they possess intense selection on the weed species, which in turn are controlled efficiently. As a result, only the resistant individuals are allowed to pass their genes to the next generation (Jasieniuk *et al*., 1996).

In Malaysia, paraquat resistance was detected in *Crassocephalum crepidoides* (Benth.) S. Moore

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1990 in the potato and sweet peas fields of Tanah Rata, Cameron Highlands (Itoh *et al.,* 1990). Ismail *et al.* (2001) discovered that the resistant biotype is approximately 3-fold more tolerant than the susceptible counterpart. The first case of the glyphosate-resistant annual grassy weed, *Eleusine indica* (L.) Gaertn was reported in orchards of Teluk Intan in 1998. The level of resistance in the resistant biotype in Teluk Intan was found to be 8 to 12-fold higher than the susceptible biotype (Lim and Ngim, 2000).

There has also been reported herbicide resistance cases in the state of Pahang, Perak, Johor, Kedah, Penang and Melaka (Heap, 2005). These herbicide resistance cases were found in vegetables farms, rice fields, oil palm plantations and rubber plantations. Almost all the herbicide-resistant weed species detected at these areas have developed resistance towards acetolactate synthase (ALS) inhibitor herbicides, paraquat, synthetic auxin herbicides or glyphosate (Heap 2005).

Hedyotis verticillata Lam., commonly known as woody borreria, is a broadleaf weed with dark green leaves, rarely branched. It is a creeper belonging to the family, Rubiaceae (Lim, 1997), and is usually found in open areas in the lowlands (Barnes and Chan, 1990). *Hedyotis verticillata* is one of the noxious perennial weeds, that has assumed to be importance in oil palm plantations recently. Once the weed matures, its stems turn woody, making it difficult to control (Ong and Teo, 1990).

This study was conducted to investigate glyphosate and paraquat resistance in the broadleaf weed, *H. verticillata* infesting oil palm plantations in Terengganu, and the degree of resistance in the resistant biotypes.

MATERIALS AND METHODS

Seed sources

Seeds of *Hedyotis verticillata* were collected from three study sites in the oil palm plantations of Terengganu namely the Land Consolidation and Rehabilitation Authority (FELCRA) plantation at Bukit Sudu near Kuala Terengganu, the FELCRA Bukit Kapah plantation, near Kuala Berang and the Federal Land Development Authority (FELDA) plantation at Belara, Kuala Terengganu. From each location, 30 plants with dry and mature seeds of the putative herbicide-resistant and susceptible biotypes were collected in response to herbicide performance. Flowers from each plant were placed inside an envelope and labeled individually.

Herbicides

Herbicides used were paraquat (Venger®) containing 250 g ai/L solution and glyphosate (Roundup Transorb®) containing 410 g ai/L solution.

Seed processing and seed sowing

Sandpaper was used to scarify the seed coat before sowing. Seeds of 18 plants of the putative herbicide-resistant and susceptible biotypes were germinated in 108 cells 28 cm x 56 cm seedling trays containing soil potting mixture (Vriezenven®) and kept in the greenhouse at 29 ± 6 ºC, 12 hour photo period and at light intensity of 800 μEm^{-2} s^{-1}. Plants were watered twice daily to ensure the soil was moist. Three to five days after germination, six seedlings of each biotype from each parent plant were transplanted into seedling trays for screening.

Screening for herbicide resistant (R) and susceptible (S) biotypes

Two to three days after transplanting, seedlings of each biotype with 3 to 4 leaves were sprayed with paraquat or glyphosate at the recommended rate of 0.75 or 0.72 kg a. i. ha^{-1}, respectively, using a compression sprayer (Matabi Style 7), delivering 450 L ha^{-1} at 200 kPa.

Three to four weeks after spraying, plants that survived the recommended dosage were considered the R biotypes and plants that died at recommended dosage were considered to be of the S biotypes. The R seedlings that survived the herbicide treatment and the untreated S seedlings were grown in isolation to prevent cross-pollination. The R and S biotype seeds collected from the first generation were used in the subsequent studies.

Dose-response experiments

Seed of the R and S biotypes of *H. verticillata* were germinated in the greenhouse in 28 x 56 cm trays containing commercial soil potting mixture. Three to five days after sowing, seedlings of both biotypes were transplanted into seedling trays containing soil potting mixture. The plants were watered twice daily and fertilized with 1g organic fertilizer, once weekly. Three to four days after transplanting, both the R and S biotypes were sprayed with paraquat or glyphosate at the 3 to 4 leaf stage, with a compression sprayer as described previously. The doses of paraquat and glyphosate used were 0, 0.01, 0.03, 0.09, 0.27, 0.81, 2.43 and 7.29 kg a. i. ha^{-1}

The seedlings were then randomized and arranged according to the factorial design. Seedlings from each biotype were randomly divided into seven or eight herbicide rate

treatment groups, with five to ten plants per treatment. Three to four weeks after spraying, plants were harvested and shoot fresh weight was determined. Data were fitted to a logistic regression model as follows (Seefeldt *et al.*, 1995; KUK *et al.,* 2002)

$$Y = c + [(d-c) / 1 + (x/x_0)^b)] \text{ or } Y = d / [1 + (x /x_0)^b]$$

Where Y = fresh weight of plants harvested, c and d = the coefficients corresponding to the lower and upper asymptotes, b = the slope of the line, x_0 = the herbicide rate to inhibit shoot growth by 50%, x = the herbicide dose. Regression analyses were conducted and herbicide rates to inhibit plant growth by 50% (ED_{50}) were calculated from the regression equations. The resistance index was calculated as ED_{50} of the R biotypes divided by the ED_{50} of the S biotypes.

RESULTS AND DISCUSSION

Table 1 shows herbicide resistance cases detected in *Hedyotis verticillata* from three different locations in Terengganu, to glyphosate and/or paraquat at the 3 to 4-leaf stage in the greenhouse. Several biotypes of *H. verticillata* were found to be resistant to one or two herbicides used in oil palm plantations. *Hedyotis verticillata* from oil palm plantations of FELCRA Bukit Kapah and FELDA Belara have developed multiple-resistance towards glyphosate. In the oil palm plantations of FELCRA Bukit Sudu, *H. verticillata* was only found to be resistant to paraquat.

In oil palm plantations of FELCRA Bukit Kapah, glyphosate, metsulfuron-methyl, and paraquat have used for more than three consecutive years while triclopyr was only used since 2002. Herbicide combinations such as paraquat plus triclopyr, glyphosate or metsulfuron-methyl to control weed populations have been practiced rotationally for two to three years since 2002. Biological control using livestock like cattle is also one of the methods used to control weed population in plantations (Muhamad, personal communication, 2004).

Table 1. Glyphosate and/or paraquat resistance in *Hedyotis verticillata* obtained from oil palm plantations at three locations in Terengganu

Location	District	Herbicide resistance case
FELCRA Bukit Sudu	Kuala Berang	Paraquat
FELCRA Bukit Kapah	Kuala Berang	Glyphosate Paraquat
FELDA Belara	Kuala Terengganu	Glyphosate Paraquat

Hedyotis verticillata from Bukit Kapah was found to have multiple-resistance to both glyphosate and paraquat at approximately four-fold (Figure 1) and two-fold (Figure 2) level of resistance as compared to the susceptible biotypes. The occurrence of glyphosate and paraquat resistance may be due to repeated application of these herbicides for more than three consecutive years. Application of herbicides can kill the population of weeds but not all of weeds are removed. Prolific seed production by the resistant plants rapidly shifts the population towards the resistant biotypes. High seed production coupled with genetic variation increases the probability that resistance will evolve (Gressel, 2002). This is because each weed species has a particular genetic makeup and herbicide resistance genes are considered to be present in the wild populations but at very low level (Valverde *et al.,* 2000). Frequent use of a herbicide will expose the weed population to selection pressure which may lead to an increase in the number of surviving resistant individuals in the population. Consequently, the resistant weed population may increase to the point that adequate weed control cannot be achieved by the application of the said herbicide.

Herbicide combination can be beneficial in weed control and will also delay the occurrence of resistance cases to the different herbicides applied in combination (Powles, 1997). However, tank mixtures of paraquat plus glyphosate, metsulfuron or triclopyr applied does not help prolong the occurrence of paraquat and glyphosate resistance in *H. verticillata* at the oil palm plantations of FELCRA Bukit Kapah because this herbicide combination was practiced for not more than three years, implying paraquat and glyphosate resistance may have occurred in these plantations before tank mixing paraquat with glyphosate, metsulfuron or triclopyr began.

In oil palm plantations of FELDA Belara, several herbicides namely metsulfuron-methyl, paraquat and glyphosate have been used to control the weed populations. All of these herbicides have been used continuously since they were introduced commercially in the market in the 1980's. In addition, herbicide combinations have never been practised at this location (Gani and Zakaria, personal communication, 2004). *Hedoyotis verticillata* from this location has also developed multiple-resistance towards glyphosate and paraquat. Based on the ED_{50}, *H. verticillata*

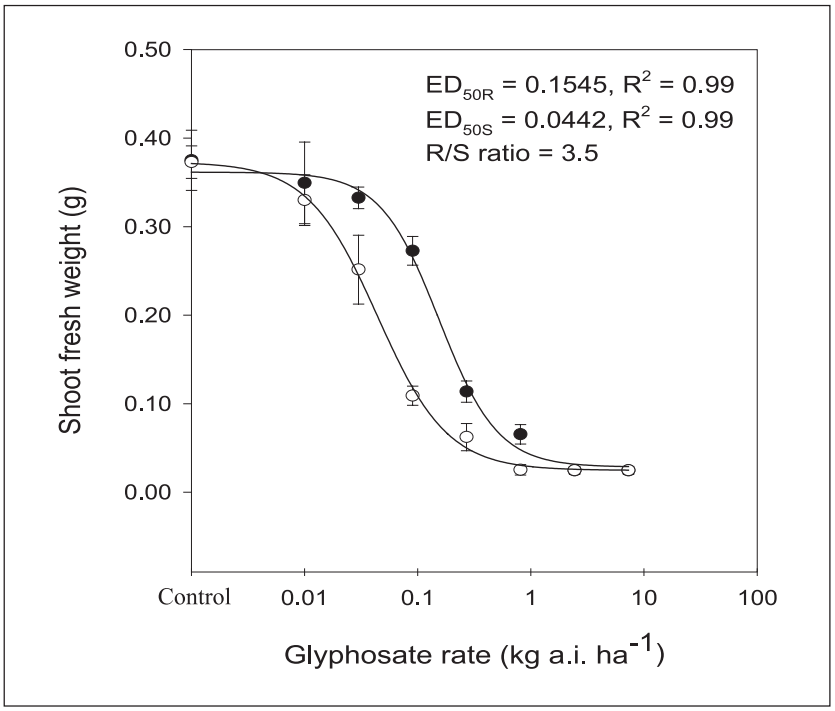

Fig. 1. Shoot fresh weight of the susceptible (o) and resistant (•) biotypes of *Hedyotis verticillata* collected from the oil palm plantation at FELCRA Bukit Kapah as affected by glyphosate in whole-plant assay. The herbicide was applied at the 3 to 4-leaf stage and shoot fresh weight was determined 21 days after treatment. Vertical bars represent standard deviation of the mean.

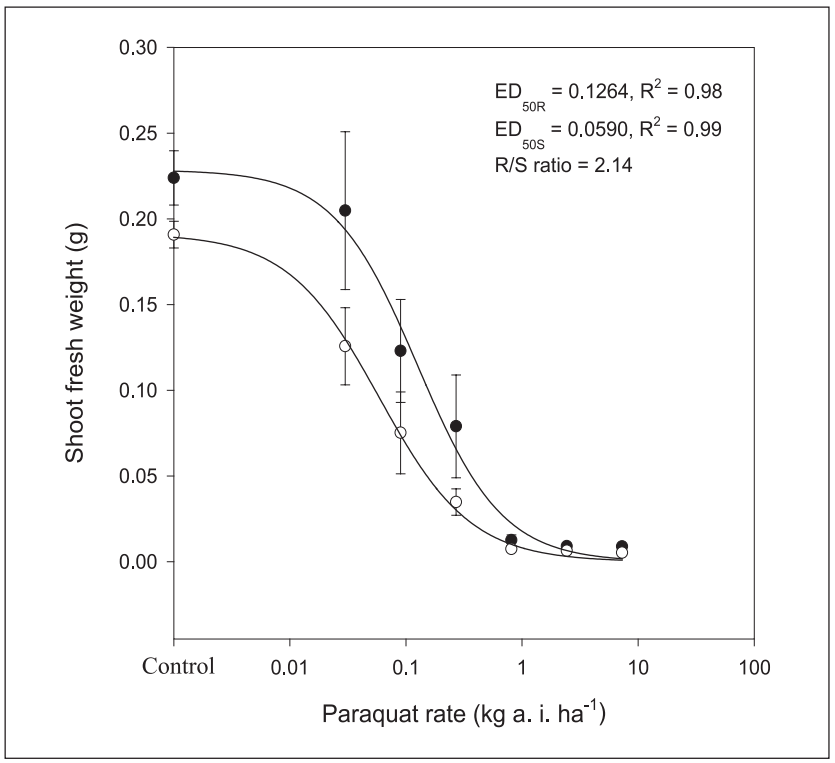

Fig. 2. Shoot fresh weight of the susceptible (o) and resistant (•) biotypes of *Hedyotis verticillata* collected from the oil palm plantation at FELCRA Bukit Kapah as affected by paraquat in whole-plant assay. The herbicide was applied at the 3 to 4-leaf stage and shoot fresh weight was determined 7 days after treatment. Vertical bars represent standard deviation of the mean.

was shown to be resistant to glyphosate and paraquat with approximately 2-fold (Figure 3) and 3-fold (Figure 4) difference in the ED_{50} as compared to the susceptible biotypes. Applying the same herbicide for a long period without tank mixtures may have contributed to glyphosate and paraquat resistance in *H. verticillata.*

Several herbicides namely paraquat, glyphosate, metsulfuron-methyl, triclopyr, fluroxypyr, 1-methyl heptyl ester and picloram are being used to control weed populations in oil palm plantations of FELCRA Bukit Sudu. Tank mixtures of paraquat and metsulfuron-methyl were applied to control *H. verticillata* beginning in the year 2000 (Zaini, personal communication, 2004). Only the paraquat-resistant biotypes of *H. verticillata* was detected with approximately 2–fold (Figure 5) difference in the ED_{50} between the resistant and susceptible biotypes at this location. This may be due to the frequent use of paraquat since the 1980's. Continuous use of herbicides with a similar mode of action will inevitably lead to resistance (Matthews, 1994). Control of *H. verticillata* using herbicide combinations of paraquat and metsulfuron-methyl may not be able to remove this weed successfully because applying paraquat will keep on increasing the selection pressure towards the paraquat-resistant biotype of *H. vercillata*. It has been proposed that for a mixture to be efficacious in resistance management, both herbicides in combination should be able to control the same spectra of weeds (Wrubel and Gressel, 1994). No glyphosate resistance cases were found at this location. Infrequent application of glyphosate (Zaini, personal communication, 2004) may be the main factor responsible for suppressing the occurrence of glyphosate-resistant biotypes of *H. verticillata* at FELCRA Bukit Sudu.

The results of dose-response tests clearly showed that several glyphosate-resistant and paraquat resistant biotypes of *Hedyotis verticillata* from the oil palm plantations of FELDA Belara, FELCRA Bukit Sudu and FELCRA Bukit Kapah with 2 to-4 fold resistance levels have been identified. The history of herbicide usage and weed control approaches such as tank mixture, single herbicide and herbicide rotation may have contributed to different types of resistance cases occurring at the three locations studied. Based on this study, suitable herbicides with different modes of action from glyphosate and paraquat could be employed to effectively control the resistant-biotypes of *H. verticillata*. Nevertheless, practices

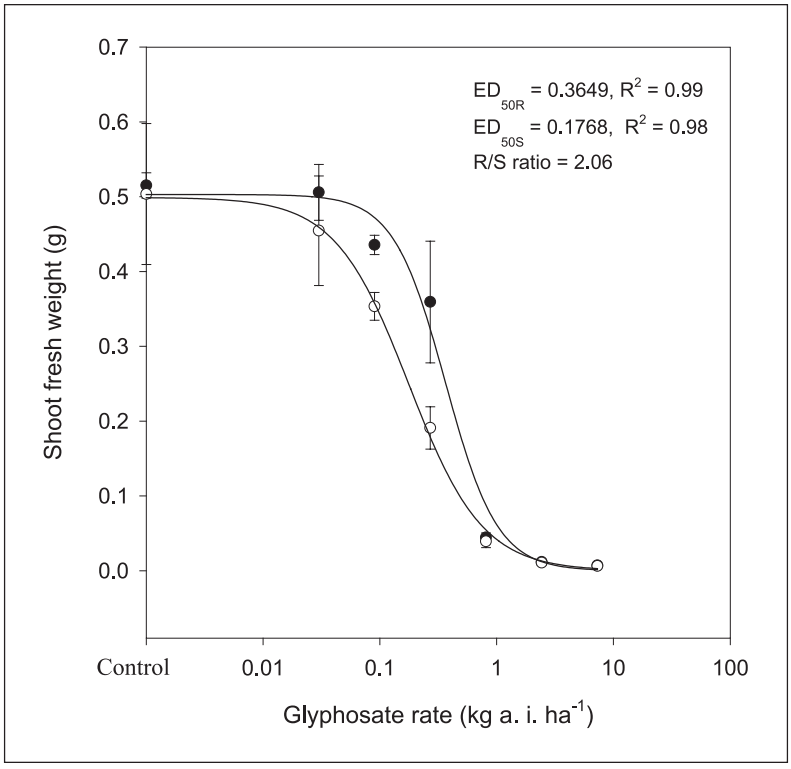

Fig. 3. Shoot fresh weight of the susceptible (o) and resistant (•) biotypes of *Hedyotis verticillata* collected from the oil palm plantations at FELDA Belara as affected by glyphosate in whole-plant assay. The herbicide was applied at the 3 to 4-leaf stage and shoot fresh weight was determined 21 days after treatment. Vertical bars represent standard deviation of the mean.

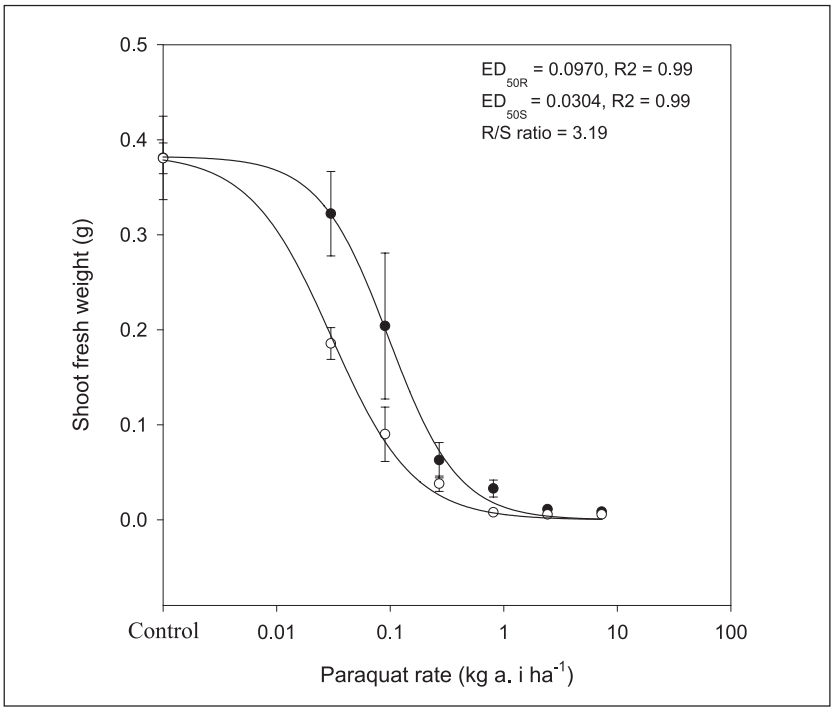

Fig. 4. Shoot fresh weight of the susceptible (o) and resistant (•) biotypes of *Hedyotis verticillata* collected from the oil palm plantation at FELDA Belara as affected by paraquat in whole-plant assay. The herbicide was applied at the 3 to 4-leaf stage and shoot fresh weight was determined 7 days after treatment. Vertical bars represent standard deviation of the mean.

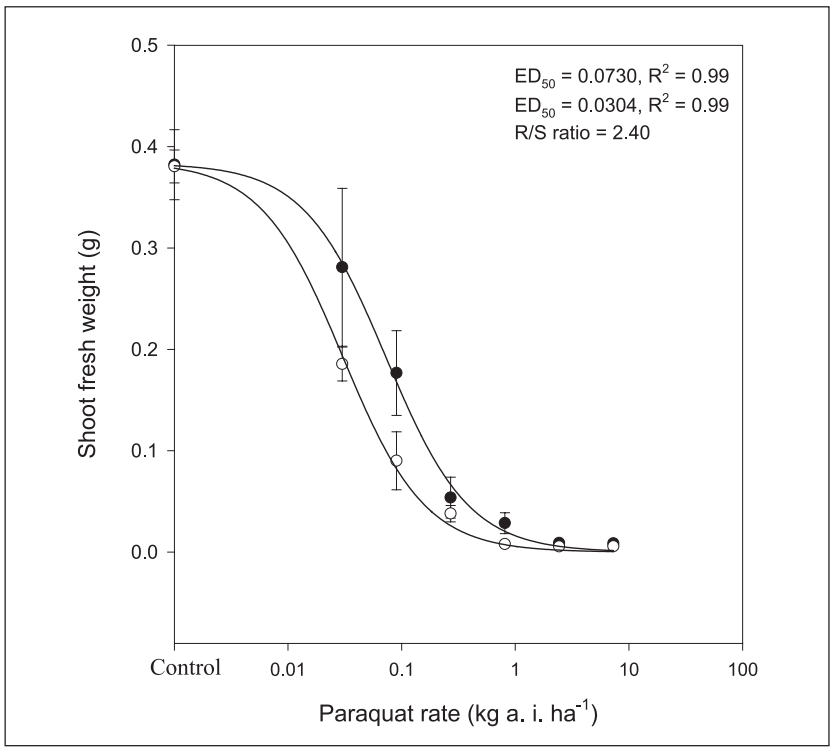

Fig. 5. Shoot fresh weight of the susceptible (o) and resistant (•) biotypes of *Hedyotis verticillata* collected from the oil palm plantation at FELCRA Bukit Sudu as affected by paraquat in whole-plant assay. The herbicide was applied at the 3 to 4-leaf stage and shoot fresh weight was determined 7 days after treatment. Vertical bars represent standard deviation of the mean.

of herbicide rotation and tank mixtures are recommended when applying suitable herbicides to control *H. verticillata* in oil palm plantations in order to delay or preclude the occurrence of resistance cases in the future.

ACKNOWLEDGEMENT

This research was supported by KUSTEM fundamental research grant 54152.

REFERENCES

Barnes, D.E. & Chan, L.G. 1990. *Common Weed of Malaysia and Their Control.* Ancom Berhad, Shah Alam.

Gressel, J. 2002. *Molecular biology of weed control*. Taylor & Francis, New York.

Heap, I. M. 2005. International survey of herbicide resistant weeds [online]. USA: Weed Science Society of America, http://www.weedscience.org/in.asp

Humburg, N.E., Colby, S.R., Lym, R.G., Hill, E.R., McAroy, W.J., Kitchen, L.M. & Prasad, R. 1989. *Herbicide Handbook of the Weed Science Society of America, 6th ed.* Champaign, Illinois: Weed Science Society of America.

Ismail, B.S., Chuah, T.S. & Hussin, K.H. 2001. Paraquat resistance in the broadleaf weed *Crassocephalum crepidioides* from the Cameron Highlands, Malaysia. *Plant Protection Quarterly*, **16(1):** 44-46.

Itoh K, Azmi, M, Ahmad, A. 1990. Paraquat resistance in *Crassocephalum crepidioides*, *Amaranthus lividus* and *Conyza sumatrensis* in Malaysia. In: *Proceeding of the Third Tropical Weed Science Conference*. Lee, S.A & Kon, K.F. (Eds.), Malaysian Plant Protection Society, Kuala Lumpur. pp. 489-493.

Jasieniuk, M.A, Brule-Babel, A.L & Morrison, I.N. 1996. The evolution and genetics of herbicide resistance in weeds. *Weed Science,* **44**: 176-193

Kuk, I.Y., Kwon, O.O., Jung, H.I., Burgos, N.R. & Guh, O.J. 2002. Cross-resistance pattern and alternative herbicides for *Rotala indica* resistant to imazosulfuron in Korea. *Pesticide Biochemistry and Physiology,* **74**: 129-138.

Lim, F.W. 1997. *Reproductivity and seed germination characterictics of Woody Borreria (Diodia ocmifolia) in a plantation ecosystem.* M. Sc. thesis. Universiti Putra Malaysia, Selangor, Malaysia. 71 pp.

Lim, L.J. & Ngim, J. 2000. A first report of glyphosate-resistant goosegrass (*Eleusine indica* (L) Gaertn) in Malaysia. *Pesticide Management Science,* **56:** 336-339.

Matthews, J.M. 1994. Management of Herbicide Resistant Populations. In: *Herbicide Resistant in Plants : Biology and Biochemistry.* Powles,S.B. & Holtum, J.A.M. (Eds.). Lewis Publishers. Boca Raton, Florida, USA. pp. 1-25.

Monaco, T.J, Weller, S.C. & Ashton, F.M. 2002. *Weed Science: Principle and Practice, 4th ed.* John Wiley & Sons, Inc, New York.

Ong, K.P. & Teo, L. 1990. Bioefficacy of herbicides for *Hedyotis verticillata* control. In: *Proceeding of the Third Tropical Weed Science Conference*. Lee, S.A & Kon, K.F. (Eds.), Malaysian Plant Protection Society, Kuala Lumpur. pp.167-175.

Powles, S.B. 1997. Success from adversity: herbicide resistance can drive changes to sustainable weed management systems. In: *Proc. Brighton Crop Protection Conference – Weeds,* **3:** 119-1126. British Crop Protection Council:Brighton.

Seefeldt, S. S., Jensen, J. E. & Fuerst, E. P. 1995. Log-logistic analysis of herbicide dose-response relationship. *Weed Technology,* **9:** 218-227.

Valverde, B.E., Riches, C.R. & Caseley, J.C. 2000. *Prevention and Management of Herbicide Resistant Weed in Rice: Experiances from Central America with Echinochloa colona.* Costa Rica: Camara de Insumos Agropecuarios.

Wrubel, R. P. & Gressel, J. 1994. Are Herbicide Mixtures Useful for Delaying the Rapid Evolution of Resistance? A Case Study. *Weed Technology,* **8:** 635-648.