Paraquat resistance in the broadleaf weed *Crassocephalum crepidioides* (Benth.) S.Moore from the Cameron Highlands, Malaysia

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Summary

A paraquat-resistant broadleaf weed, *Crassocephalum crepidioides* infesting tomato and cabbage fields in Tanah Rata, Cameron Highlands, Malaysia was identified in a greenhouse study. Paraquat dichloride at 1 kg ha^{-1} had been applied twice a year to the affected fields for 10 years. Dose-response tests were conducted, using 6- and 9-week-old plants that had received repeated exposure to paraquat and those that had no history of paraquat application. The estimated ED_{50} (paraquat dose required to reduce shoot fresh weight by 50%) of both biotypes was lower for the 6-week-old as compared to the 9-week-old plants. Furthermore, the ED_{50} of resistant (R) biotypes was approximately three times higher than that of the susceptible (S) biotype for both the growth stages studied.

Introduction

Shortly after the introduction of herbicides for weed control, Harper (1956) predicted that repeated use of the same herbicide would almost inevitably be followed by the development of a resistant biotype of the weed. In 1979, the first case of a paraquat-resistant weed (*Conyza bonariensis*) was reported in Egypt (Summer 1980). About 16 years later, 18 dicotyledonous and seven monocotyledonous weed biotypes had developed resistance to paraquat due to frequent application of the herbicide in 12 countries (Heap 1994, 1997). Paraquat-resistant biotypes have been reported to withstand from five to 250 times higher dosage of paraquat than susceptible biotypes (Purba *et al.* 1993). Paraquat has a single target and a specific mode of action: characteristics which contribute to the high probability for evolution of herbicide resistance (Holt and LeBaron 1990).

A paraquat-resistant biotype of the annual broadleaf weed *Crassocephalum crepidioides* was first reported in 1990 in the tomato fields near Tanah Rata, Cameron Highlands, Malaysia (Itoh *et al.* 1990). *C. crepidioides*, also known as redflower ragleaf, is a common weed in tomato and potato fields in the Cameron Highlands. Paraquat had been applied twice a year at a concentration of 1 kg ha^{-1} to these fields for ten years. Preliminary studies had shown that the level of resistance in the R biotype of *C. crepidioides* was 100-fold higher than that of the S biotype through leaf disc tests involving various concentrations of paraquat (Itoh *et al.* 1990). The objective of this preliminary study was to determine the degree of resistance in *C. crepidioides* by conducting whole plant dose response tests on the R and S populations under greenhouse conditions.

Materials and methods

Plant materials

Crassocephalum crepidioides seeds and leaves were collected from two populations in the Cameron Highlands, Pahang. One population was derived from five patches (M1, M2, M3, M4 and M5) in tomato and cabbage fields in the research station of the Malaysian Agricultural Research and Development Institute (MARDI), at Tanah Rata where paraquat had been applied twice a year for ten years. Another population came from five patches (B1, B2, B3, B4 and B5) along the roadsides in Brinchang and was presumed not to have been previously exposed to paraquat. The weed populations from Brinchang were located 15 km from the MARDI research station.

Leaves of plants from the five patches, at the MARDI Research Station and Brinchang were collected and screened for resistance to paraquat by punching 5 mm leaf discs and floating them on various concentrations of paraquat: 0, 0.1, 1.0, 10, 100, 1000 ppm under light at 25°C for 48 hours (Itoh *et al*. 1990). Five discs from each leaf and ten leaves from each patch were used for testing each concentration of paraquat. Each treatment was replicated three times. Paraquat was applied as a formulated product of Gramoxone® (CCM).

Seeds from each patch were germinated in the 36 × 26 × 5 cm plastic trays containing moist sand. After two weeks, seedlings of each patch were transplanted into 7 cm diameter cups containing sand and grown in the greenhouse at 29 ± 4°C, a 12 hour photoperiod and a light intensity of 800 µE m^{-2} sec^{-1} PPFD. Plants were watered twice daily and fertilized with 5 mL of half strength Hoagland's nutrient solution three times weekly. Three weeks after transplanting, seedlings were screened for resistance to paraquat by spraying with paraquat at the rate of 0.5 kg ha^{-1} in order to confirm resistance or susceptibility.

Dose-response experiments

The seeds were selected from the identified R and S populations as confirmed by the two tests and then germinated separately as described above. After two weeks, seedlings of the R and S biotypes were transplanted into 7 cm diameter cups containing sand and grown in the greenhouse at 29 ± 4°C, a 12 hour photoperiod and a light intensity of 800 µE m^{-2} sec^{-1} PPFD. The plants were watered twice daily and fertilized with 5 mL of half strength Hoagland's nutrient solution three times weekly. Plants from both biotypes were sprayed with paraquat at 6 and 9 weeks after germination.

Plants from each biotype were randomly divided into six herbicide treatment groups, with nine plants per treatment. Paraquat doses used were 0, 0.25, 0.50, 1.00, 2.00 and 4.00 kg ha^{-1}. The herbicide was applied to the plants with a knapsack sprayer positioned 60 cm above the plants, delivering 450 L ha^{-1} at one bar pressure . After spraying, the plants from each biotype were placed in the dark for 12 hours, and then returned to the greenhouse. The plants were arranged in a completely randomized design in each experiment. Three days after spraying, the above-ground living tissue remaining on each plant was harvested and weighed.

Statistical analysis

The shoot fresh weight was plotted against the paraquat rate in order to determine the paraquat dose that caused a 50% reduction in shoot fresh weight (ED_{50}). The dose was expressed in kg ha^{-1}. Data from each biotype was fitted to the exponential decay function: $Y = a + e^{b+cx}$ where a, b and c are coefficients, and x is the paraquat rate. All data was subjected to analysis of variance and means were compared using the Duncan test and t-test at the 5% level of significance. The Duncan test was used to compare the means obtained from using different paraquat rates on each biotype while the t-test was used to compare ED_{50} between R and S biotypes at both growth stages for the dose-response experiments

Results and discussion

Leaf disc tests and whole plant assays

Leaf disc test on leaf samples from Brinchang showed that plants from three patches (B1, B2 and B3) were susceptible to paraquat while leaf samples from all five patches in MARDI were resistant to paraquat. The results of the whole plant test showed that plants grown from seeds

collected from four patches, with the exception of M5 were resistant to paraquat, while all plants grown from seeds collected from Brinchang were susceptible to paraquat.

Results from the resistance screening of the whole plant were different from that involving the leaf disc technique, probably due to environmental factors such as soil nutrients and the degree of shading which were not homogeneous under field conditions. An earlier study had shown that plants grown under a high nitrogen regime manifested significantly less paraquat damage than plants grown in soil with low nitrogen levels (Lutman *et al.* 1975). This could probably be the reason when using the leaf disc test, the leaves from patch M5 maintained their green colour even at higher concentrations of paraquat.

The leaves collected from patches B4 and B5 were exposed in the sunlight while the leaves from patches B1, B2 and B3 were collected from shady places. Exposed conditions may be the cause of the leaves from patches B4 and B5 having thicker cuticle and higher trichome density than leaves from the three other patches, resulting in lower uptake of paraquat. Hence, the result of the leaf disc assay showed that leaf discs from patches B1, B2 and B3 had completely lost their green colour at 10 ppm while leaf discs from patches B4 and B5 only showed complete green discoloration at 1000 ppm.

However, the seeds collected from the five patches at the MARDI Research Station and Brinchang were germinated and grown under the same environmental conditions in the greenhouse, the difference in leaf characteristics and other environmental factors were reduced. Therefore, the whole plant screening showed that patches M1, M2, M3 and M4 are the R biotype while the patch M5 from MARDI and plants from all five pathes at Brinchang are the S biotype.

However, there are several methods that can be used to detect and monitor herbicide-resistant weeds, some of which include bioassay techniques and biochemical tests. Biochemical methods are not only able to detect resistance at lower frequencies than those obtained from bioassays, but they are more convenient and permit the level of resistance to be quantified without the need to test several samples at different doses (Brent 1986). However, biochemical methods should be used with caution, since resistance depends on alternative mechanisms to the method under test and could be missed (Truelove and Hensley 1982).

Response of 6-week-old seedlings of C. crepidioides to paraquat

Figure 1 shows the dose-response curves of R and S biotypes at 6 weeks. The relationship between *C. crepidioides* shoot fresh weight and paraquat rate can be described by an exponential decay function. Shoot fresh weight of both biotypes decreased as the paraquat dose increased, but the S biotype appeared to have more rapid decline in shoot fresh weight compared to the R biotype. However, at higher doses of paraquat (>2 kg ha^{-1}), both R and S biotypes had more than 85% reduction in shoot growth. Furthermore, the decreased shoot fresh weight of both biotypes with the application of paraquat at the rate of 2 kg ha^{-1} did not differ significantly (P>0.05) from the rate of 4 kg ha^{-1}, suggesting no apparent difference in phytotoxicity of paraquat at the rate of 2 kg ha^{-1} and 4 kg ha^{-1} towards *C. crepidioides*. Both biotypes were susceptible at the recommended rate to paraquat at 6 weeks, but the S biotype was more susceptible than the R biotype. The estimated ED_{50} of the S biotype was only 1/10 the recommended application rate of 1 kg ha^{-1} while the estimated ED_{50} of the R was approximately 1/3 the recommended rate, giving a 2.9-fold difference in ED_{50} between R and S biotypes (Table 1).

Response of 9-week-old seedlings of C. crepidioides to paraquat

Figure 2 presents the dose-response curves of the R and S biotypes at 9 weeks. The shoot fresh weight of both biotypes decreased exponentially as the paraquat rate increased. There was no significant difference in the decreased shoot fresh weight for both biotypes between the rate of 0.25 kg ha^{-1} and the control (0 kg ha^{-1}), suggesting that paraquat at the rate of 0.25 kg ha^{-1} was not significantly phytotoxic to *C. crepidioides* as compared to the control plants. However, significant differences were shown in decreased shoot fresh weight between the R and S biotypes at all higher rates of paraquat (0.25–4 kg ha^{-1}). At the highest dose of paraquat (4 kg ha^{-1}), the R biotype showed reduction in shoot growth of 72% while the S biotype had 93% reduction in fresh weight. Generally, the plants were more tolerant to paraquat treatment at 9 weeks as compared to 6 weeks. Relatively, the S biotype was more susceptible, with the ED_{50} slightly lower than the recommended application rate. In contrast, the ED_{50} of the R biotype was approximately 2.4 times the

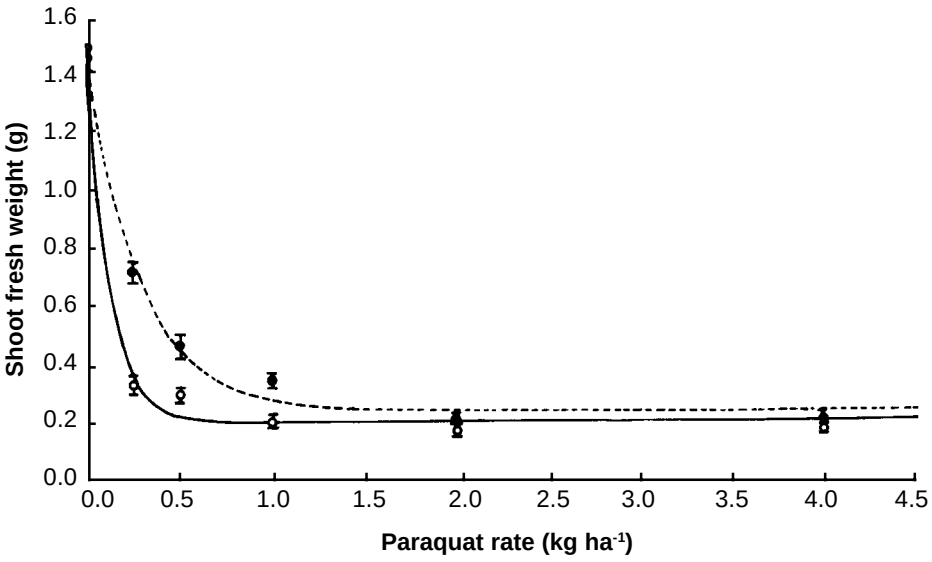

Figure 1. Effects of paraquat concentrations on the shoot fresh weight of 6-week-old seedlings of *C. crepidioides*. ● R biotype, ○ S biotype.

Table 1. ED_{50} estimates from dose response curves[A] for *C. crepidioides* shoot fresh weight of 6- and 9-week-old plants.

Growth stage	ED_{50}[B] (kg a.i. ha^{-1})	
	R	S
6 weeks	0.2922 (0.0089) a	0.1004 (0.0085) b
9 weeks	2.3902 (0.1470) a	0.9176 (0.0507) b

[A] Data from R and S populations were fitted to exponential function.
[B] ED_{50}: paraquat dose that causes a 50% reduction in shoot fresh weight.
Means within rows with similar letters of each growth stage are not significantly at the 5% level by t-test. SE of means are given in parentheses.

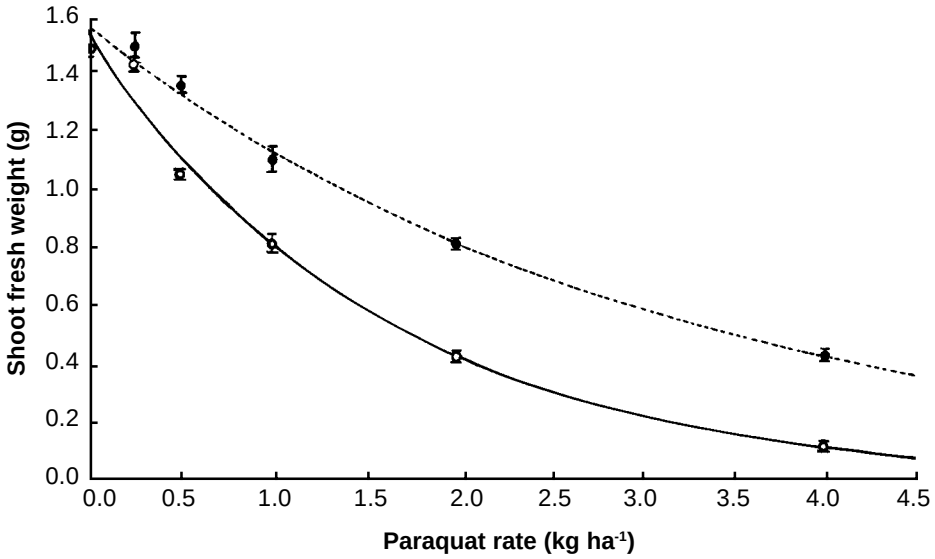

Figure 2. Effects of paraquat concentrations on the shoot fresh weight of 9-week-old seedlings of *C. crepidioides*. ● R biotype, ○ S biotype.

recommended rate, giving a 2.6-fold difference in resistance between the R and S biotypes (Table 1).

Both biotypes of *C. crepidioides* showed lower ED_{50} at 6 weeks as compared to 9 weeks, suggesting that paraquat was more effective on 6-week-old plants. This may be due to their younger developmental stage (Alida *et al.* 1998, Ewert 1964). Generally, a young plant has less epicuticular wax as well as cuticle compared to the mature plant. Hence, more paraquat may be absorbed into young plants of *C. crepidioides* because of less epicuticular wax and cause rapid death. Furthermore, unpublished data from the Universiti Kebangsaan Malaysia (UKM) laboratory demonstrated that the plant had lower superoxide dismutase (SOD) and peroxidase (POD) levels at 6 weeks in comparison to 9 weeks. These antioxidant enzymes play an important role in eliminating toxic oxygen generated by the paraquat treatment (Babbs *et al.* 1989, Shaaltiel *et al.* 1988). A lower level of SOD and POD in the plant at the younger age may have caused them to fail in removing superoxide and hydrogen peroxide rapidly enough, thereby resulting in death.

It was reported earlier that the level of the resistance in resistant biotypes of *C. crepidioides* was 100-fold higher than that of the susceptible biotypes (Itoh *et al.* 1990). This study was based on leaf discs that were dipped into various concentrations of paraquat. This method involved lateral as well as vertical uptake of paraquat in leaf discs while absorption of paraquat into leaves through bioassay tests was rather limited. Therefore, the lower resistance ratios (approximately three) found in the present study may be due to difference in the type of assay used (Alida *et al.* 1998).

Acknowledgments

This work was supported by the research grant IRPA 08-02-02-0004. We express our appreciation to Ms. Samiah Kadri, Mr. Kalithasan Kailasam and Mr. Chong Tet Vun for their technical assistance.

References

Alida, S., Colleen, D., Martha, J. and Susan, J. (1998). Paraquat resistance in horseweed (*Conyza canadensis*) and Virginia pepperweed (*Lepidium virginicum*) from Essex Country, Ontario. *Weed Science* 46, 200-4.

Babbs, C.F., Pham, J.A. and Coolbaugh, R.C. (1989). Lethal hydroxyl radical production in paraquat-treated plants. *Plant Physiol*ogy 90, 1267-70.

Brent, K.J. (1986). Detection and monitoring of resistant forms: an overview. *In* 'Pesticide resistance. Strategies and tactics for management', pp. 298-312. (National Academy Press, Washington).

Ewert, A. (1964). Susceptibility: Factors in the plant modifying the response of a given species to treatment. *In* 'The physiology and biochemistry of herbicides', ed. L.J. Audus, pp. 401-22. (Academic Press Inc., London).

Harper, J.L. (1956). The evolution of weeds in relation to resistance to herbicides. Proceedings of the 3rd British Weed Control Conference, pp. 215-33.

Heap, I.M. (1994). Identification and documentation of herbicide resistance. *Phytoprotection* 75, 85-90.

Heap, I.M. (1997). The occurrence of herbicide-resistant weeds worldwide. *Pesticide Science* 51, 235-43.

Holt, J.S. and LeBaron, H.M. (1990). Significance and distribution of herbicide resistance. *Weed Technology* 4, 141-49.

Itoh, K., Azmi, M. and Ahmad, A. (1990). Paraquat resistance in *Crassocephalum crepidioides*, *Amaranthus lividus* and *Conyza sumatrensis* in Malaysia. Proceedings 3rd Tropical Weed Science Conference, eds S.A. Lee and K.F. Kon, pp. 489-93. (Malaysian Plant Protection Society, Kuala Lumpur).

Lutman, P.J.W., Sagar, G.R., Marshall, C. and Headford, D.W.R. (1975). The influence of nitrogen status on susceptibility of segments of cereal leaves to paraquat. *Weed Research* 15, 89-95.

Purba, E., Preston, C. and Powles, S.B. (1993). Paraquat resistance in a biotype of *Vulpia bromoides* (L.). *Weed Research* 33, 409-13.

Shaaltiel, Y., Chua, N.H., Gepstein, S. and Gressel, J. (1988). Dominant pleiotropy controls enzymes co-segregating with paraquat resistance in *Conyza bonariensis*. *Theoretical Applied Genetics* 75, 850-56.

Summers, L.A. (1980). 'The bipyridinium herbicides'. (Academic Press, London).

Truelove, B. and Hensley, J.R. (1982). Methods of testing for herbicide resistance. *In* 'Herbicide resistance in plants', eds H.M. LeBaron and J. Gressel, pp. 565-72. (John Wiley and Sons, New York).