IDENTIFICATION OF PARAQUAT-RESISTANT *SOLANUM NIGRUM* AND *S. AMERICANUM* BIOTYPES

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ABSTRACT

Sweetpotato (*Ipomoea batatas* (L.) Lam.) plants are highly susceptible to weed competition during their early field establishment period. For a long time, the contact herbicide paraquat has been widely used within the sweetpotato production system, often as the only herbicide applied to control weeds that emerge within the crop. A low rate of paraquat is applied repeatedly over the crop each season, eliminating seedling weeds at a very young stage, until the crop canopy provides adequate competition. However, the application of paraquat to selectively destroy seedling weeds has inadvertently selected for paraquat-resistant weed biotypes. This study confirms growers' field observations that populations of paraquat-resistant solanaceous weeds are now common, identifying them as *Solanum nigrum* L. and *S. americanum* Mill. Paraquat cannot be used selectively when these resistant biotypes are present, as the concentrations required to control the weed species would be lethal to the crop itself.
Keywords: herbicide resistance, *Solanum nigrum*, *S. americanum*, *Ipomoea batatas,* sweetpotato, kumara.

INTRODUCTION

Plants of sweetpotato (*Ipomoea batatas* (L.) Lam.), known locally as kumara, are propagated on a commercial scale by sprouting storage roots retained from the previous season. The crop is established in the field by hand-transplanting the sprouts, which are un-rooted as they are cut above the propagation media to minimise disease transfer and root distortion. The planting process must allow for the warm temperatures required due to the subtropical nature of the crop, late cultivation to maximise soil aeration for hand-planting and frequent watering until new roots form, all of which optimise weed germination. Growers in the Dargaville-Ruawai region have had increasing difficulty in controlling the weed black nightshade (*Solanum nigrum* L.) within the sweetpotato cropping system. This region of northern New Zealand is a well established sweetpotato production area, growing over 94% of the national crop (StatisticsNZ 2000).

Generally, low rates of the contact herbicide paraquat are applied repeatedly over the crop during early sweetpotato field establishment, until the sweetpotato plants are capable of forming adequate ground cover. Each paraquat application destroys successive batches of newly emerged seedling weeds, leaving the hardier sweetpotato transplants relatively unharmed (Lewthwaite & Triggs 2000). Paraquat acts in the presence of light, so sweetpotato growers apply the herbicide at dusk or early dawn to improve its selectivity for weeds within the crop. The industry has been heavily reliant on paraquat for over 37 years (Coleman 1972).

A herbicide-resistant black nightshade biotype has previously been found within New Zealand pea crops of the Manawatu region. However, this biotype was resistant to triazine herbicides, which are photosystem II inhibitors (Harrington et al. 2001). The herbicide paraquat is a bipyridylium, which works as a photosystem I inhibitor (Heap 2009).

Internationally, paraquat resistance has been found across various plant species (Heap 2009). Amongst solanaceous plants, paraquat resistance has been reported in *S. nigrum* within Malaysian vegetable crops in 1990 (Itoh et al. 1992), while paraquat resistance in *S. americanum* Mill. has been reported in USA tomato crops (Bewick et al. 1990; Chase et al. 1998).

This paper explores the relationship between paraquat application rates and plant responses for *Solanum* weed populations derived from commercial sweetpotato fields.

MATERIALS AND METHODS

Mature berries were collected from solanaceous weeds in commercial sweetpotato fields of the Dargaville-Ruawai region. The plants were identified to species level as *S. nigrum* and *S. americanum*. For comparison purposes, seed of these two species was also obtained from non-sweetpotato production areas of the greater Auckland region, *S. nigrum* from Pukekohe and *S. americanum* from Waitakere. The seed was extracted from the berries then chilled at 5ºC for 3 weeks, to break dormancy.

The treated seed was sown into trays of commercial peat/pumice potting mix and maintained in a greenhouse. Following germination and cotyledon development, the seedlings were transplanted into pots of growing media, just as the first true leaf became visible. The pots were tissue culture dishes, 8.5 cm in diameter and 2.3 cm deep, which were modified by inserting four drainage holes. The nine plants transplanted into each pot were spread to maximise interplant distance, with eight plants around the pot circumference and one plant in the centre. Spray treatments were applied when plants had developed their first true leaf and the second true leaf was commonly just visible. Following spray treatment, the plants were maintained at the Pukekohe Research Centre in an unheated greenhouse under natural lighting, until living plants showed five true leaves, at which time the numbers of dead plants were recorded.

Paraquat was applied in the Gramoxone® 250 formulation (250 g ai/kg), with application concentrations adjusted through the addition of distilled water. Each specimen pot was individually treated, with the treatments applied down a vertical spray tube 30 cm high and 9 cm in diameter. A spray mist was generated within the top of the tube by a hand-operated spray bottle, delivering a foliar spray to runoff of 0.011 ml/cm^2 (equivalent to a field water rate of 1100 litre/ha).

Experiment 1

The Pukekohe region is located well away from the main sweetpotato production area with its associated high paraquat usage, so a *S. nigrum* seed population collected from Pukekohe (P1) was used to construct a standard paraquat concentration response curve. Each treatment was replicated across 20 pots of P1 plants, so that 180 individual plants were exposed to each concentration. Initially, paraquat treatments were applied at six rates: 0, 0.0009, 0.0086, 0.0291, 0.0870 and 0.2174 g ai/litre (equivalent to field rates from 0.99 to 239.14 g ai/ha). Based on the initial plant response, a further eight rates of paraquat, 0.0026, 0.0088, 0.0121, 0.0163, 0.0184, 0.0200, 0.0239 and 0.0400 g ai/litre (equivalent to field rates from 2.86 to 44.00 g ai/ha), were applied under comparable conditions. Curves were fitted (GenStat 2003) to both data sets and a common response curve constructed for population P1.

Experiment 2

Solanum spp. seed populations collected from sweetpotato fields at Dargaville-Ruawai and from fields without sweetpotato at Pukekohe were evaluated under a single paraquat application rate (0.040 g ai/litre, equivalent to a field rate of 44 g ai/ha); a rate selected using the data obtained in Experiment 1. Each population treatment was replicated across nine pots of plants. The seed populations included the standard Pukekohe *S. nigrum* population (P1), a yellow-berried mutant form (later identified as *S. nigrum* L. f. *humile* (Willd.) Lindm.) also from Pukekohe (P2), and five Dargaville-Ruawai populations from commercial sweetpotato fields, designated DR1 to DR5. One of the Dargaville-Ruawai seed populations (DR4) was later identified as *S. americanum*, while the remaining four populations consisted of *S. nigrum*. The data were analysed (GenStat 2003) and mean responses calculated.

Experiment 3

A *S. nigrum* seed population collected from Dargaville-Ruawai (DR3) was selected on the basis of Experiment 2, to construct a paraquat concentration response curve for paraquat-resistant plants. Each concentration treatment was replicated across 20 pots. Initially, paraquat was applied at 10 rates: 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56, 5.12 and 10.24 g ai/litre (equivalent to field rates from 22 to 11,264 g ai/ha). Based on the initial plant response, a further eight treatments of paraquat (0.04, 0.64, 0.96, 1.28, 2.56, 5.12, 10.24 and 15.36 g ai/litre, equivalent to field rates from 44 to 16,896 g ai/ha) were applied. Curves were fitted (GenStat 2003) to both data sets and a common response curve constructed for population DR3.

Experiment 4

A *S. americanum* seed population sourced from Dargaville-Ruawai (DR4) was identified as paraquat-resistant in Experiment 2. Paraquat concentration response curves were constructed for *S. americanum,* based on a seed population sourced from Waitakere (W1) as an example of a population outside the sweetpotato production area, and for seed population DR4 as an example of a population from within the commercial sweetpotato production region. For the DR4 population, each treatment was replicated across 20 pots, apart from the lowest concentration, which was replicated across nine pots. The six rates of paraquat applied were 0.040, 0.16, 0.64, 2.56, 10.24 and 15.36 g ai/litre (equivalent to field rates from 44 to 16,896 g ai/ha). For the W1 population, each treatment was replicated across six pots, so that 54 individual plants were exposed to each paraquat concentration. The five rates of paraquat applied were 0.010, 0.020, 0.040, 0.16 and 0.64 g ai/litre (equivalent to field rates from 11 to 704 g ai/ha). Curves were fitted (GenStat 2003) to the data generated by each population.

RESULTS AND DISCUSSION

The project identified two *Solanum* spp. that appear to be relatively common weeds of the sweetpotato crop in the Dargaville-Ruawai region, *S. nigrum* and *S. americanum*. The species *S. nigrum* is an exotic weed that is widely distributed throughout New Zealand and is generally recognised by its common name ‘black nightshade’. The species *S. americanum* is presumed indigenous to New Zealand, although it may have been introduced by pre-European Maori (Webb et al. 1988). Although *S. americanum* is not generally differentiated from *S. nigrum* for commercial crop management purposes, it is known by the common name ‘small-flowered nightshade’ and can be readily distinguished in the field by its relatively small corolla diameter.

Sweetpotato growers in the Dargaville-Ruawai region became increasingly aware that their standard weed management techniques, based on the herbicide paraquat, were not controlling *S. nigrum*. Growers typically apply paraquat over the establishing crop at 100 g ai/ha (Lewthwaite & Triggs 2000). A survey conducted on a cultivated sweetpotato field with a history of poorly controlled *S. nigrum* estimated that this species comprised 46.9% of the weed composition (S.L. Lewthwaite, unpubl. data), a much higher level than the 1.1% previously observed in a non-problematic field with a similar cropping background (Lewthwaite & Triggs 2000). If allowed to mature to flowering stage, *S. nigrum* plants from the poorly controlled field tended to display a more compact growth habit than those from other areas.

As growers varied in their field experiences with controlling *S. nigrum*, the project developed a diagnostic paraquat concentration, capable of distinguishing standard and paraquat-resistant *S. nigrum* seedling populations. A *S. nigrum* seed population (P1) collected from outside the sweetpotato production region was used to construct a concentration response curve (Fig. 1).

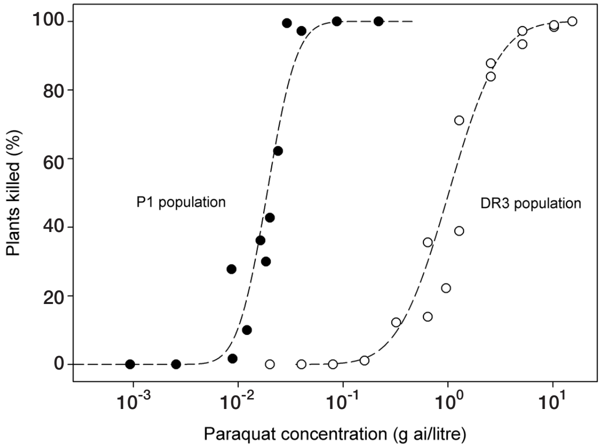

FIGURE 1: Fitted response curves for percentage seedling *Solanum nigrum* deaths following foliar application of the herbicide paraquat at increasing concentrations. The two seed populations presented are P1 from a non-sweetpotato production region (closed circles) and DR3 from a commercial sweetpotato field (open circles).

Lethal Dose (LD) thresholds were calculated from the fitted curve, to estimate paraquat concentrations that gave 50, 95 and 99% plant death rates (Table 1). A diagnostic paraquat concentration of 0.040 g ai/litre (equivalent to a field rate of 44 g ai/ha) was then used to evaluate a number of *Solanum* spp. seedling populations for resistance. The seed populations sourced from outside the sweetpotato production area responded as expected, with high mean death rates of 98.9 and 97.8% for populations P1 and P2 respectively. The berries of surviving P2 plants remained yellow-green when mature, rather than turning black, indicating that the population consisted of the common variant form *S. nigrum* L. f. *humile* (Willd.) Lindm. The remaining five seed populations were sourced from sweetpotato fields, but gave differing responses. The DR1 population responded in a similar manner to P1 and P2, with a mean death rate of 96.7%. However, the mean death rates of the remaining four populations were extremely low: DR2 (1.1%), DR3 (1.1%), DR4 (0.0%) and DR5 (3.9%). When plants surviving this diagnostic test were grown to maturity, it was found that all the DR populations were *S. nigrum*, apart from DR4, which was *S. americanum*.

A *S. nigrum* population (DR3) identified as paraquat-resistant by the diagnostic rate was then used to construct a further paraquat concentration response curve (Fig. 1). The fitted curves for standard (P1) and resistant (DR3) populations were of similar shape, with the resistant population response being at higher paraquat concentrations. LD thresholds were calculated from the fitted curve, providing estimated paraquat concentrations for 50, 95 and 99% plant death rates in a resistant population (Table 1). A comparison of estimates for the LD_{99} concentration between the standard and resistant *S. nigrum* populations indicates that a concentration increase of approximately 100-fold is required to produce a similar response in the resistant population.

Paraquat concentration responses curves were also constructed for a *S. americanum* population from outside the sweetpotato production area (W1) and for the resistant population (DR4) identified using the *S. nigrum* derived diagnostic rate. A comparison of the fitted curves (Fig. 2) for the standard and resistant *S. americanum* populations show they have the same general shape, but with the resistant population requiring much higher paraquat concentrations to produce a similar response. LD thresholds were calculated from each fitted curve, providing estimated paraquat concentrations for 50, 95 and 99% plant death rates in both standard (W1) and resistant (DR4) populations (Table 2). A comparison of estimates for the LD_{99} concentration between the standard and resistant *S. americanum* populations indicates a concentration increase of more than 18-fold is required to produce a similar response in the resistant population.

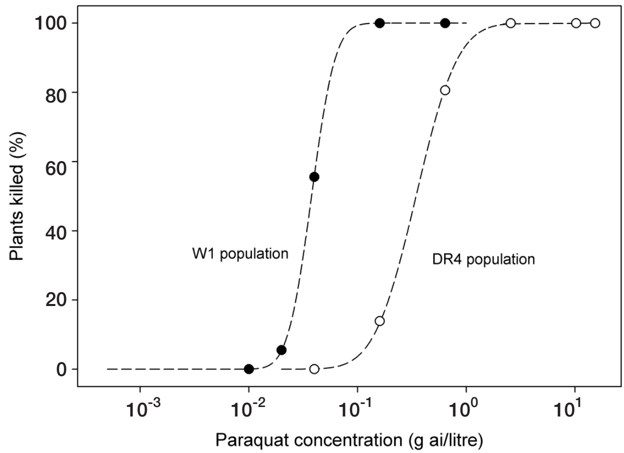

FIGURE 2: Fitted response curves for percentage seedling *Solanum americanum* deaths following foliar application of the herbicide paraquat at increasing concentrations. The two seed populations presented are W1 from a non-sweetpotato production region (closed circles) and DR4 from a commercial sweetpotato field (open circles).

TABLE 1: Estimates of the paraquat concentration required for lethal dose (LD) thresholds of 50, 95 and 99% plant death in seedling *Solanum nigrum* populations. Standard errors (SE) and 95% confidence levels are given. The two seed populations presented are P1 sourced from a non-sweetpotato production region and DR3 from a commercial sweetpotato field.

Population	LD	Estimate g ai/litre	SE g ai/litre	Lower 95% g ai/litre	Upper 95% g ai/litre
P1					
	50	0.019	0.0004	0.018	0.020
	95	0.044	0.0019	0.040	0.048
	99	0.062	0.0035	0.056	0.070
DR3					
	50	1.47	0.051	1.38	1.57
	95	4.5	0.32	4.0	5.2
	99	7.1	0.68	6.0	8.8

Comparing the two *Solanum* species, based on the estimates of LD_{99} for the paraquat resistant populations, the paraquat resistant *S. nigrum* population (Table 1) requires more than a 4-fold increase in concentration to produce a death rate similar to that of the resistant *S. americanum* population (Table 2). In mixed field populations of these paraquat resistant biotypes, applications of suitably high paraquat rates would selectively eliminate the species *S. americanum,* while potentially leaving a viable *S. nigrum* population, a reversal of the paraquat species selectivity indicated by mean LD_{99} estimates for standard biotypes.

TABLE 2: Estimates of the paraquat concentration required for lethal dose (LD) thresholds of 50, 95 and 99% plant death in seedling *Solanum americanum* populations. Standard errors (SE) and 95% confidence levels are given. The two seed populations presented are W1 sourced from a non-sweetpotato production region and DR4 from a commercial sweetpotato field.

Population	LD	Estimate g ai/litre	SE g ai/litre	Lower 95% g ai/litre	Upper 95% g ai/litre
W1					
	50	0.038	0.0704	0.034	0.044
	95	0.072	0.1686	0.058	0.111
	99	0.095	0.2188	0.071	0.167
DR4					
	50	0.35	0.056	0.31	0.38
	95	1.08	0.099	0.91	1.34
	99	1.73	0.129	1.39	2.31

There are few herbicides identified as suitable for use in sweetpotato production, so paraquat is often the only herbicide applied to control weeds within the crop. Despite the identification of paraquat-resistant *Solanum* weed populations, paraquat is still fundamental to New Zealand sweetpotato production and usage patterns remain comparable to those of the past. The development of further herbicide resistant weed populations will be promoted by a number of issues. There is generally little crop rotation, as sweetpotato fields are left fallow over winter or converted to pasture for a period ranging from a single winter to several seasons. *Solanum* spp. are relatively common annual weeds within the crop and are capable of producing large quantities of seed. The repetitive application of a single herbicide, with its solitary mode of action, within and across seasons, effectively removes all susceptible weeds at the seedling stage. However, weeds that remain, despite exposure to the herbicide, mature to become a breeding population of resistant plants, unless removed manually or by cultivation. The use of particularly low paraquat application rates, to discriminately impair weed but not crop growth, further facilitates selection for resistance (Neve & Powles 2005).

New Zealand sweetpotato growers are interested in developing new effective weed management strategies that inspire public confidence and conform to good agricultural practice, an approach that is consistent with current international interest in selecting ‘reduced risk’ pesticides for sweetpotato production. Paraquat has been manufactured commercially since 1961, but has drawn public controversy despite its established efficacy as a herbicide (Senarathna et al. 2009). The debate is primarily centred on paraquat’s involvement in cases of lethal poisoning, sometimes accidental but mainly through deliberate ingestion in cases of self harm. As a consequence, the international use of paraquat tends to be highly regulated or banned, and is subject to negative publicity. While in the future the crop may be managed with minimal herbicide usage (Treadwell et al. 2007), in the meantime a wider portfolio of herbicides with different modes of action is required, to enable growers to control weed populations without compounding the existing resistance issues.

CONCLUSIONS

In conclusion, the field application of paraquat sprays to selectively control seedling weeds within the sweetpotato cropping system has inadvertently selected for paraquat-resistant biotypes of the weeds *S. nigrum* and *S. americanum*. This is the first formal report of paraquat resistance within the New Zealand weed flora. A combination of factors, such as prolonged reliance on a single herbicide, repetitive use within cropping seasons, low application concentrations and the localised production area appear to have produced significant populations of the resistant biotypes. The paraquat concentrations required to control the resistant *Solanum* biotypes, even at a very early seedling stage, would be lethal to the sweetpotato crop itself.

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