Cleancrop™ Brassica System: The development of herbicide resistant brassica crops for New Zealand farming systems

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Abstract

This paper presents results from a breeding program which, using seed mutagenesis combined with traditional plant breeding techniques, has resulted in the development of the Cleancrop™ Brassica system. Seedlings of *Brassica napus* with increased chlorsulfuron resistance were identified following seed mutagenesis with ethyl methanesulfonate (EMS) and *in vitro* screening of seedlings in the presence of the herbicide. Surviving herbicide resistant seedlings were used in a traditional breeding program to introgress resistance into leafy turnip, bulb turnip, rape and swede. Acceptable herbicide resistance to at least a double application of chlorsulfuron from either a pre-emergence or a 4-leaf post emergence timing has now been produced in all these crops. Results from trials sown at Lincoln, Canterbury and Knapdale, Southland with chlorsulfuron herbicide application at both these timings with HT-S57 swede showed excellent weed control and no noticeable crop phytotoxicity. The pre-emergence herbicide application produced significantly more total dry matter per hectare than the untreated control. This weed management system represents a new tool for New Zealand farmers which will expand the use of forage brassica crops into more marginal areas which historically have had difficult to control weed problems.

Keywords: Seed mutagenesis, chlorsulfuron, acetolactate synthase, field plant breeding, Cleancrop™ Brassica System, HT Brassica™

Introduction

Forage brassica crops are needed to fill periods of feed shortages in winter, when pasture growth is restricted due to low temperatures, and in summer due to drought, and to improve the nutritive value of the total diet at times when pasture quality is low (White & Hodgson 1999). Forage brassica crops occupy the largest area of cultivated land in New Zealand, with over 300 000 hectares grown annually. About 30–40% of the area is grown for the dairy industry, with much of this crop used as winter feed (kale, swedes and turnips) in the South Island and summer milking feed in the North Island (bulb turnips and to a lesser extent rape). The other 60–70% of the area consists of winter, summer and autumn feed crops (kale, swedes, rape, bulb turnip and leafy turnip) for the sheep, beef and deer industries (PGG Wrightson Seeds pers. comm.).

The aim of forage brassica crops is to achieve high dry matter (DM) yields which can be utilised efficiently by grazing animals. The DM yield and forage quality of forage brassicas can both be significantly reduced through competition by weeds. Cultivation to kill young weeds prior to sowing and the use of chemical weed control in the early growth stages of the crop is usually essential for a successful crop (White & Hodgson 1999).

Currently in New Zealand there are only a few herbicides registered for use in forage brassicas, and none provide an ideal solution to weed control. Drawbacks include only partial control of the targeted weed spectrum, poor performance in dry conditions, undesirable levels of phytotoxic effects on the brassica crop, and high levels of residual toxicity in the soil affecting species such as clover and lucerne. Internationally, plants with improved herbicide resistance have been developed using traditional plant breeding and selection, as well as through transgenic methods (Conner & Meredith 1989; Field *et al*. 1993). The development of plants with resistance to sulfonylurea herbicides by seed mutagenesis has been successful in a number of plant species (Conner *et al*. 1994) and is recognised as being developed through non-transgenic technology by the Environmental Risk Management Authority (ERMA).

Sulfonylurea herbicides are more environmentally acceptable than many conventional herbicides because of their low usage rates (application rate of chlorsulfuron (DuPont™ Telar®) is 20 g product/ha) and low mammalian toxicity (Beyer *et al*. 1988; Stidham 1991). The sulfonylurea group of herbicides kill weeds by inhibiting the activity of acetolactate synthase (ALS), the first enzyme in the biosynthetic pathway of branched chain amino acids (Ray 1984). Chlorsulfuron resistance is due to point mutations resulting in amino acid changes that reduce herbicide binding to the ALS enzyme (Yu 2003). A single primary site of action makes it easier to develop crop cultivars with greater resistance to a chosen herbicide in the sulfonylurea group (Dastgheib & Field 1995).

This paper reports the use of seed mutagenesis and traditional plant breeding for the development of forage brassicas with resistance to DuPont™ Telar® (75% chlorsulfuron), a sulfonylurea herbicide.

Seed Mutagenesis

A population of rapid cycling rape was obtained from the Crucifer Genetics Cooperative, and two lines of *Brassica napus* with resistance to chlorsulfuron were developed through seed mutagenesis. Seeds were soaked overnight (16 hours) in 0.3% ethyl methanesulfonate (EMS), washed in running water for 2–3 hours, then dried on absorbent paper for 2 days. Seeds were sown in a greenhouse and transplanted out as 33 first generation (M1) populations of 100 plants each, which were allowed to self-pollinate and set seed. The seed from each M1 population was harvested together to give 33 second generation (M2) populations of 180–2580 seeds, resulting in a total M2 population size of 30 609 seeds; this process was continued until the M4 generation, with each generation screened for chlorsulfuron resistance (Conner *et al*. 1994).

The M3 progeny of rape plants 19 and 30 showed clear segregation for seedlings that were chlorsulfuron resistant or susceptible (Table 1). As expected, resistant plants were either homozygous, true breeding plants or heterozygotes that segregated 3:1 for resistance. The observed number of homozygous and heterozygous plants was consistent with the 1:2 ratio expected for a single dominant mutation conferring resistance (Conner *et al*. 1994). The growth of lines 19c and 30a was compared to the original wild type under glasshouse conditions. All lines showed similar growth when untreated with herbicide. The wild type plants showed severe damage with all chlorsulfuron rates (Table 2). In contrast, lines 19c and 30a showed herbicide tolerance, with all plants maintaining active growth following chlorsulfuron application as high as 30 g product/ha (Conner *et al*. 1994). Some phytotoxicity was observed in both chlorsulfuron resistant lines, but was more evident in line 19c (Conner *et al*. 1994). The better of the two lines, line 30a, was chosen for use in a conventional breeding and selection programme.

Table 1. Segregation of chlorsulfuron resistance among the progeny of the rape mutants (Conner *et al*.1994)

	Number of seedlings resistant	Number of seedlings sensitive
M3 Generation		
Wild type	0	151
19	53	23
30	56	28
M4 Generation		
Wild type	0	166
19a	37	16
19b	26	10
19c	149	0
19d	80	35
30a	150	0
30b	93	0
30c	55	14
30d	54	16

Table 2. Visual assessment scores of rape lines 4 weeks after spraying with different rates of chlorsulfuron, 1 = dead, 10 = healthy (Conner *et al*., 1994)

Assessment	Line	Chlorsulfuron Concentration (g product/ha)					
		5	10	15	20	25	30
Visual Score	Wild type	2.8	2.0	1.4	1.6	1.4	1.8
SEM =0.5	19c	6.4	7.2	5.0	5.8	5.2	5.0
	30a	9.8	8.8	6.8	6.2	6.8	5.6

Plant Breeding

The plant breeding process involved several generations of backcrossing and single plant selection to introgress chlorsulfuron resistance into leafy turnip (*Brassica rapa)*, bulb turnip (*B. rapa* var. *rapa)*, forage rape (*B. napus* var. *biennis*) and swede (*B. napus* var. *napobrassica*) with enhanced forage agronomic characteristics (increased forage yield, multiple regrowth, palatability, insect and disease tolerance).

The transfer of chlorsulfuron resistance from rape (*B. napus*) to kale (*B. oleracea* var. *acephala*) is a technical challenge in breeding. This is partly due to the normally incompatible cross between these two species requiring embryo rescue to produce interspecific hybrid plants. Embryo rescue involved removing intact ovules from the plant and culturing them on a plant culture medium designed to promote growth. Plants which were successfully produced tended to revert back to more of a rape-type plant, resulting in unusable progeny. Rape is an allotetraploid species derived from a cross between the turnip and kale groups. Therefore, even if rape × kale hybrids were successfully backcrossed to kale, they would still need a recombination event between genomes to transfer the resistance to kale. The success of this cross seems very unlikely, so work is on-going with EMS seed treatment to produce mutants in an elite kale.

Between 2002 and 2009 a series of screening trials was carried out each year to test potential chlorsulfuron resistant breeding lines against a range of application rates at three growth stages (pre-emergence, 2-leaf stage and 4+-leaf stage). The aims of these trials was to ensure that all breeding material was resistant to at least twice the standard herbicide application, to ensure crop safety during spray overlaps.

From this work it was shown that chlorsulfuron application to 'Pasja' leafy turnip, 'Green Globe' bulb turnip, 'Goliath' rape and 'Aparima Gold' swede at all application rates resulted in complete crop death. In contrast, the phytotoxicity seen on 'HT-LT46' leafy

Table 3. Plant health visual scores for HT-S57 swede sown at Lincoln, Canterbury on the 10 February 2012 with 20 or 40 g product/ha of chlorsulfuron applied pre-emergence and post-emergence at the 2- or 4-leaf growth stage compared to the untreated control

	Date of Scoring		
Clorsulfuron Treatment	**03 March 2012**	**12 April 2012**	**03 May 2012**
Untreated	9.0	9.0	9.0
Pre emergence 20g/ha	9.0	9.0	9.0
Pre emergence 40g/ha	9.0	9.0	9.0
Post 20g/ha 2-leaf stage	5.8	5.8	9.0
Post 40g/ha 2-leaf stage	4.0	4.3	8.0
Post 20g/ha 4-leaf stage	8.8	8.8	9.0
Post 40g/ha 4-leaf stage	8.3	8.0	8.3
Mean	7.5	7.7	8.8
LSD (5%)	0.71	0.80	0.28
CV%	6.4	7.1	2.2

Scores are on a 1 to 9 basis (1= severe phytotoxicity, 9 = no phytotoxic symptoms)

Table 4. Leaf yield, dry matter (DM) content and plant weight of HT-S57 swede sown at Lincoln, Canterbury on the 10 February 2012 with 20 or 40 g product/ha of chlorsulfuron applied pre-emergent or post-emergent at the 2- & 4-leaf stage compared to the untreated control, harvested on the 24 April 2012 (74 days after sowing).

Chlorsulfuron Treatment	**Leaf Yield (kg DM/ha)**	**Plant Dry Matter (% DM)**	**Average Plant dry matter (g DM/plant)**
Untreated	4446	10.0	77.5
Pre-emergence 20g/ha	4202	10.4	89.0
Pre-emergence 40g/ha	4657	9.7	109.0
Post 20g/ha 2-leaf stage	4044	9.9	69.0
Post 40g/ha 2-leaf stage	3086	9.7	60.2
Post 20g/ha 4-leaf stage	4399	10.2	65.5
Post 40g/ha 4-leaf stage	4276	9.8	85.9
Mean	4159	10.0	79.4
LSD (5%)	1004	1.22	38.0
CV%	13.6	6.9	26.9

turnip after a double application rate was acceptable for a commercially grown crop. This trial result provided the confidence to enter 'HT-LT46' into commercial seed production during 2006.

The phytotoxicity from a double rate of chlorsulfuron applied to 'HT-R24' rape was of concern however. Even though plants did recover to a certain extent over time, there was too much phytotoxicity and some plant death, which was considered to be commercially unacceptable. Plants surviving after a double dose of chlorsulfuron were reselected for increased herbicide resistance, successfully retested and entered into commercial seed production in 2007. This type of plant breeding and agronomy was also carried out prior to commercial seed production for the 'HT-BT35' bulb turnip (commercial seed production in 2008) and the 'HT-S57' swede (commercial seed production 2011). Acceptable resistance to a double application rate of the herbicide chlorsulfuron has now been produced in all these crops.

Product Development

'HT-S57' swede was the focus of the product development programme over the 2011/12 summer growing season. This was due to the high value of the crop as a winter forage source and corresponding weed infestation problems currently experienced by swede growers.

Two trials were established, the first trial examined the effect of chlorsulfuron applications at different growth stages on 'HT-S57' swede from a late planting (10 February 2012) at Lincoln, Canterbury to determine the relationship between increased crop phytotoxicity and herbicide applications during a period of reducing soil temperatures. This trial also explored the effect of chlorsulfuron on 'HT-S57' at different growth stages (pre-emergence, 2- and 4-leaf stage). Plant health scores were taken and leaf yield was measured 74 days after sowing (half the recommended growing period of 150 days).

The trial was sown after peas; plots were 1.5 × 8.0 m and drilled in 150 mm drill rows at a depth of <10 mm using a Øyjord cone seeder at 1 kg/ha. Leaf yield was assessed with two randomly selected, 1 square metre quadrat cuts per plot, and total wet weight and plant number per quadrate recorded. DM was assessed by taking one bulked sample of leaf material per plot and drying at 95°C for 24 hours.

Chlorsulfuron applied at the 2-leaf stage at both the 20 and 40 g product/ha rate significantly reduced the

Table 5. Plant health and weed control visual scores of HT-S57 swede sown at Knapdale, Southland on the 1 December 2011.

	Plant Health Scores		Weed Control	
Chlorsulfuron Treatment (20 g product/ha)	**16 February 2012**	**28 March 2012**	**16 February 2012**	**28 March 2012**
Pre-emergent only	9.0	8.8	7.3	7.8
Post-emergent only	7.5	8.3	8.0	7.8
Pre- & post-emergent	6.8	6.5	8.8	8.5
Untreated	9.0	9.0	1.8	1.3
Mean	8.0	8.0	6.4	6.3
LSD (5%)	1.13	0.8	0.7	0.9
CV%	8.8	6.2	6.5	9.2

Scores 1= severe phytotoxicity/poor weed control, 9= no phytotoxic symptoms/complete weed control
Pre-emergent applied on 6/12/2011, post-emergent applied on 16/2/2012, pre-emergent + post-emergent applied on 6 December 2011 and 16 February 2012 respectively

Table 6. Yield of HT–S57 swede sown at Knapdale, Southland on the 1 December 2012, with 20 g product/ha of chlorsulfuron applied at either the pre-emergent, post-emergent (fourth leaf stage), or both stages, harvested on the 14 June 2012.

Chlorsulfuron Treatment (20 g product/ha)	Bulb Yield (kg DM/ha)	Leaf Yield (kg DM/ha)	Total Yield (kg DM/ha)	Plant Density (plants/m²)
Pre-emergent only	11995	3508	15503	7.6
Post-emergent only	9673	2926	12599	7.1
Pre- & post-emergent	9486	3210	12696	7.7
Untreated	8520	2827	11347	7.3
Mean	9918	3118	13036	7.0
LSD (5%)	3595	965	3979	2.4
CV%	22.7	19.3	19.1	20.4

kg DM/ha = kilograms of dry matter per hectare plant health score on the 3 and 12 April 2012, but plant health score increased by the 3 May 2012 score. Only the 40 g product/ha post-emergence application at both the 2- and 4-leaf stage resulted in a reduced plant health score on the 3 May 2012 (Table 3). No phytotoxicity was observed in any of the pre-emergence applications, which were identical to the untreated control (Table 3).

Chlorsulfuron applied at 40 g product/ha at the 2-leaf stage significantly reduced 'HT-S57' leaf yield at 74 days after planting (Table 4). Pre-emergence and post-emergence application at the 4-leaf stage at both 20 and 40 g product/ha had no significant effect on 'HT-S57' leaf yield or average plant weight.

A second trial, sown on the 1 December 2011 at Knapdale, Southland, measured the effect of chlorsulfuron applied to 'HT-S57' swede at the recommended rate (20 g product/ha), at pre-emergence and post-emergence at the 4-leaf stage, or at both pre- and post-emergent application. The trial was sown at 650 g/ha after pasture, using a traditional ridger. Ridge spacings were 600 mm apart and plots were 2.4 × 8 m. DM yield was assessed on 14 June 2012 by harvesting 2.2 m² of the middle two ridges from each plot and splitting the sample into leaf and bulb. Bulb percentage DM was assessed by taking a core sample from 15 swede bulbs from each plot, and leaf percentage DM was assessed by taking one bulk sample of leaf material per plot; both leaf and bulb samples were dried at 95°C for 24 hours.

Dry conditions in Southland affected crop establishment; germination was highly staggered and this resulted in a wide variation in plant growth stages in the trial. Due to this staggered germination the post-emergent chlorsulfuron applications were delayed until the 16 February 2012. On this date a number of plants had not yet reached the 4-leaf stage when the herbicide was applied, and this may have increased the phytotoxic effect of the post-emergence application.

Chlorsulfuron applied from pre-emergence or post-emergence (4-leaf stage) had no effect on 'HT-S57' compared to the untreated control (Table 5). The only phytotoxicity observed was in the combined pre- and post-emergence treatment, which may have been due to the variation in growth stages due to the dry conditions experienced. Control of wild turnip, californian thistle, dandelions, chickweed, fathen, shepherd's purse, dock, willow weed, white clover, twin cress and spurrey (yarr) was complete in the combined pre- and post-emergent treatment and only nightshade was unaffected by the application of chlorsulfuron at both timings.

To assess the effect of chlorsulfuron on total DM yield, bulb and leaf yields were assessed at crop maturity on 14 June 2012. The pre-emergent application produced significantly more total DM per hectare than the untreated control (Table 6).

Both trials showed positive results for 'HT-S57' swede when used in conjunction with chlorsulfuron herbicide within the Cleancrop™ Brassica system.

Cultivars available with Cleancrop™ technology: The Cleancrop™ Brassica system is available in a package that includes three components: seed of selected HT Brassica™ cultivar, chlorsulfuron herbicide, and a best practice and stewardship plan. These are designed to maximise the on-farm performance with responsible weed management to minimise unintended build-up of resistant weeds. Four cultivars are currently available:

HT Swede ('HT-S57') is a high yielding, white fleshed, purple skin, main crop swede with similar clubroot and dryrot tolerance to 'Aparima Gold' and improved leaf disease tolerance (PGG Wrightson Seeds 2012).

HT Rape ('HT-R24') is a high yielding 'Goliath' type rape with improved leaf percentage and crop utilisation. Crop maturity is 90–110 days after planting and it has good regrowth ability combined with excellent winter keeping ability (PGG Wrightson Seeds 2012).

HT Bulb Turnip ('HT-BT35') is a high yielding 'Green Globe' type bulb turnip with a maturity of between 90–110 days after planting and can be used for summer, autumn or winter feeding (PGG Wrightson Seeds 2012).

HT Leafy Turnip ('HT-LT46') is a multiple graze 'Pasja' type with a maturity of between 50–70 days after planting which provides a flexible grazing option for all stock types over summer, autumn and early winter (PGG Wrightson Seeds 2012).

Conclusions

The Cleancrop™ Brassica System was developed through seed mutagenesis, combined with traditional plant breeding techniques; this is internationally recognised as non-transgenic technology.

The Cleancrop™ Brassica System provides a simple, selective control of mixed weed populations, with control of wild turnip and shepherd's purse, excellent performance in dry weather, combined with reduced crop phytotoxicity and soil herbicide residue.

Chlorsulfuron (Telar®) applied at 20 g product/ha, showed excellent weed control from either a pre-emergence or a post-emergent 4-leaf application in HT-S57 swede and had no noticeable crop phytotoxicity effect, whilst having excellent level of weed control across a range of species which are economically important to control in a forage brassica crop.

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