AGRONICO PTY LTD

Independent Agronomic Advice and Research

Gene flow from herbicide resistance GM canola to weedy relatives (*Brassica rapa*) in Tasmania

FINAL REPORT

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Comissioned by: Office of the Gene Technology Regulator

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Introduction

Brassica rapa is a close relative of *B. napus* and is the weedy relative most likely to cross with GM canola. Monitoring of weed spectrums around sites in Tasmania in 2001-2002 where GM canola had been trialed revealed two sites where *B. rapa* weeds where present. The aim of the work reported here was to determine if gene flow had occurred from GM canola to *B. rapa* identified within 1 km of the sites where the GM canola had been grown.

Materials and Methods

General Procedures

During initial visits to sites, permission was granted to gain access to all areas within 1km of the GM canola trial site. *Brassica rapa* plants were then mapped within 1km of each trial site and sprayed with the appropriate herbicide. Assessments were conducted of the effect of the herbicide on the *B. rapa* and any surviving or missed plants were recorded and resprayed. A second assessment was conducted on the resprayed *B. rapa*. A summary of the general procedures for conducting the work can be found in the description of consultancy services supplied by the Office of the Gene Technology Regulator (Appendix 1).

Trial sites

Details of the two GM canola trial sites are given in Table 1. A GPS (GARMIN *e*trex) was used to record the boundary of the GM canola trial sites (Table 2).

Table 1 Individual site details

Licence	Site no.	Code	Area	Site size (Ha)
PR62X(4)	6	AG-98-6	Cambridge	11.2
PR77X	16/17	-	Colebrook	9.9

Table 2 GPS co-ordinates and marks for the two GM canola trial sites.

Licence	GPS mark	GPS coordinates
PR62X(4)	001	532000E : 5288340N
PR62X(4)	002	531863E : 5287967N
PR62X(4)	003	531943E : 5287904N
PR62X(4)	004	532057E : 5287886N
PR62X(4)	005	532163E : 5288206N
PR62X(4)	006	532483E : 5288154N
PR62X(4)	007	532449E : 5288242N
PR77X	014	535848E : 5261622N
PR77X	015	535618E : 5261821N
PR77X	016	536200E : 5262079N
PR77X	017	536194E : 5261848N

Permission was gained from the landowners of the trial sites, and landowners with land falling within the 1 km boundary of the trial sites, prior to access. Visit logs, containing a summary of

the activities conducted at each visit, were filled in each time the sites were visited (Appendix 2).

Mapping of B. rapa

A GPS was used to locate *B. rapa* plants within 1 km of the trial site. Individual plants or areas where a large number of plants were present were marked with a white peg. Where adult plants and small numbers of emerging plants were found in an area, individual plant numbers were recorded. In areas where there were large number of plants were emerging, a 0.25 m^2 quadrat was used to estimate the number of plants present.

Herbicide applications

Mature plants and low numbers of 2-4 true leaf plants in an area were individually spot sprayed. For spot spraying with Basta at the Cambridge site, a rate of 75 ml/15 L water was used. For spot spraying with Roundup at the Colebrook site, a rate of 100 ml/15 L water was used. Where there were large numbers of 2-4 true leaf plants present at the Cambridge site, a pressurized 'PET' sprayer with a 1.5 metre wide boom and four Hardie 4110-12 fan nozzles was used. The spray width was 2 metres. The herbicide was applied at a water rate of 240 L/ha at a pressure of 200 kPa. Standard operating procedures SOP004 and SOP005 (Appendix 3) were followed for calibration of the spray unit and calculation of the walking speed required for application of Basta at a rate of 3 L/ha. Trial sites were assessed within 1-2 weeks of herbicide application for the presence of survivors. Herbicide damage was assessed on a linear scale of 1-9 (adapted from, Australian Weeds Committee (1979)) as detailed in Table 3.

Table 3 Rating scale for plant stature scores

Score	Effects
9	No effect evident
8	Negligible effect: some stunting and yellowing just visible
7	Slight effect: stunting and yellowing obvious – effects reversible
6	Substantial chlorosis and (or) stunting – most effects reversible
5	Majority of plants effected: strong chlorosis and stunting – some thinning of stand
4	Most plants damaged irreversibly: some plants killed: much necrosis and distortion
3	Severe effect: significant number of plants killed
2	Very severe effect: majority of plants killed, remainder showing necrosis and wilting
1	Total loss of plant

Trial Schedule

A schedule of the activities carried out at the sites is given in Table 4.

Table 4 Trial schedule

Date	Activity
12/07/02	Visited Colebrook and Cambridge sites and organized permission to access neighboring land
15/07/02	Mapping of *B. rapa* at the Colebrook site and spraying with Roundup
16/07/02	Mapping of *B. rapa* at the Cambridge site
22/07/02	Spraying of *B rapa* at the Cambridge site
05/08/02	Assessing *B. rapa* at the Colebrook and Cambridge sites and re-spraying

	surviving plants at the Cambridge site
12/08/02	Assessing re-sprayed plants at the Cambridge site

Results

Colebrook site

Mapping of plants

There were 11 *B rapa* plants identified in a Brassica seed crop on the Colebrook property and 3 plants identified along the roadside. The GPS co-ordinates for these are given in Table 5, along with comments on their growth stage and stature.

Table 5 Location of *B. rapa* plants within 1 km of the Colebrook GM canola site

GPS mark	GPS coordinates	Comments
012	531484E : 5288336N	15 cm tall, in flower, on the roadside
013	532039E : 5287726N	2 plants, both approx. 15 cm tall and flowering
019	532778E : 5287746N	20 cm diametre rosette *
020	532778E : 5287746N	10 cm diametre rosette *
021	532769E : 5287750N	10 cm diametre rosette *
022	532769E : 5287750N	10 cm diametre rosette
023	532744E : 5287754N	15 cm diametre rosette *
024	532773E : 5287886N	15 cm diametre rosette
025	532784E : 5287886N	35 cm diametre rosette *
026	532787E : 5287883N	25 cm diametre rosette *
027	532872E : 5287854N	55 cm diametre rosette *
028	Within 5m of 027	45 cm diametre rosette *
029	Within 2m of 028	45 cm diametre rosette*

* previous herbicide damage from Goal, stature 7.

A map of the site showing the approximate locations of the *B. rapa* plants is given in Appendix 4.

Assessment of herbicide damage

None of the *B. rapa* plants showed any resistance to Roundup. Phytotoxicity scores at the assessment on the 5th August 2002 are given in Table 6. Original data sheets can be found in Appendix 5. Photographs of the herbicide effects are given in Appendix 6.

Table 6 *B. rapa* stature scores after spraying with Roundup

GPS mark	Stature	Comment
012	2	-
013	2	Both plants with necrotic leaves and chlorotic stems
019	1	-
020	2	-
021	1	-
022	1	-
023	1	-
024	2	Severe chlorosis on newer leaves, slight chlorosis on older leaves
025	2	Severe chlorosis on newer leaves, slight chlorosis on older leaves
026	2	Severe chlorosis on newer leaves, slight chlorosis on older leaves
027	3	Severe chlorosis on newer leaves, slight chlorosis on older leaves

028	3	Severe chlorosis on newer leaves, slight chlorosis on older leaves
029	2	-

Cambridge site

Mapping of plants

There were approximately 8800 *B rapa* plants at the 2-4 TL stage and 275 plants from 10 cm rosettes to flowering that were identified and mapped. The GPS co-ordinates for these are given in Table 7, along with comments on their growth stage.

Table 7 Location of *B. rapa* plants within 1 km of the Cambridge GM canola site

GPS mark	GPS coordinates	Comments
037	534934E : 5261561N	~ 190 plants from 25 cm rosette to 1.5 m flowering
038	534943E : 5261573N	~ 117/m^2 at the 2 TL stage – area 20 m x 20 m
039	535153E : 5261677N	~ 40 in 2 m^2 area at the 2 TL stage
040	535024E : 5261229N	~ 123/m^2 at 2-4 TL stage – area 1 x 5 m
041	535543E : 5261172N	~ 36/m^2 at 2-4 TL stage – area 0.5 x 2 m
042	535516E : 5261192N	~ 21/m^2 at 2 TL stage – area 1 x 5 m
043	535533E : 5261368N	~ 206/m^2 at 2 TL stage – area 4 x 4 m
044	535354E : 5261023N	60 plants ranging from 4 TL to flowering
045	535953E : 5261243N	20 plants ranging from 10 cm rosette to flowering
046	535948E : 5261214N	3 at 10-30 cm rosettes
047	535959E : 5261267N	2 flowering plants
048	536018E : 5260994N	6 at 4-5 TL

After the initial assessment 21 plants were identified ranging from 5cm rosettes to flowering that either missed a herbicide spray or had some resistance. The GPS co-ordinates for these are given in Table 8.

Table 8 Location of healthy *B. rapa* plants identified during assessment for herbicide damage

GPS mark	GPS coordinates	Comments
049	535963E : 5261256N	4 plants 15 cm diametre rosettes
050	535959E : 5261250N	1 flowering plant
051	535953E : 5261241N	3 plants 10 cm diametre rosettes
052	535946E : 5261245N	1 flowering plant
053	535856E : 5261012N	3 plants 5-10 cm rosettes
054	534889E : 5261532N	1 flowering plant
055	534887E : 5261539N	2 plants 10 cm diametre rosettes
056	534891E : 5261543N	1 flowering plant
057	534894E : 5261548N	1 flowering plant
058	534897E : 5261551N	1 flowering plant
059	534910E : 5261559N	1 flowering plant
060	534925E : 5261563N	1 flowering plant
061	534925E : 5261563N	1 20 cm diametre plant

A map of the site showing the approximate locations of the *B. rapa* plants is given in Appendix 7.

Assessment of herbicide damage

Phytotoxicity scores at the assessment on the 5th August 2002 are given in Table 9. Phytotoxicity scores at the assessment on the 12th August 2002 are given in Table 10. Original data sheets can be found in Appendix 8. Photographs of the herbicide effects are given in Appendix 9.

Table 9 *B. rapa* stature scores after spraying with Basta on 22/07/02

GPS mark	Stature	Comment
37	2-5	-
38	2-3	-
39	1-2	-
40	3-5	-
41	3	-
42	3-4	-
43	3-4	-
44	2-4	Chlorotic leaves and stems
45	2-4	Exposed leaves with severe chlorosis. Protected leaves with less damage
46	3	Exposed leaves with severe chlorosis. Protected leaves with less damage
47	3	Severe chlorosis on older leaves. Some chlorosis on younger leaves
48	3-4	-

Table 10 *B. rapa* stature scores after spraying with Basta on 05/08/02

GPS mark	Stature	Comment
49	3	Necrotic leaf margins
50	4	-
51	2-4	-
52	3	Chlorotic upper leaves and necrotic lower leaves
53	2-3	-
54	4	-
55	3-4	Necrotic leaf margins
56	4	Chlorotic upper leaves and necrotic lower leaves
57	5	-
58	4	-
59	4	-
60	3	-
61	2	-

Conclusion

Only a limited number of *B. rapa* plants were found at the Colebrook site, which is consistent with previous monitoring records for this site. Most of the *B. rapa* plants located at this site were found in a brassica seed crop between the 400 metre and 1 km boundary from the GM canola site in the direction of the prevailing winds. Due to the low number of *B. rapa* plants found, the site did not provide a good test of the potential for the occurrence of gene flow, although no resistance

was present to Roundup in any of the plants tested. Most of remaining area surrounding the site up to the 1 km boundary is in pasture and being grazed predominantly by sheep.

The Cambridge site, in contrast to the Colebrook site, provided a good test for the potential for gene flow between the GM canola and *B. rapa*. Over 9000 plants from cotyledon stage to flowering were identified within the 1 km boundary from the GM canola site. Much of the surrounding land is either cultivated for a variety of annual crops or contains olives or grape vines. Regular cultivation of land or weed spraying around trees and vines provides an ideal environment for the establishment of Brassica weeds. Much of the Cambridge area contains significant numbers of Brassica weeds including *B. rapa.* The area is used for growing a number of Brassica seed crops and the University farm, which surrounds the Cambridge site, is used for conducting trial work on brassica crops as well as conventional canola. No resistance was found to the application of Basta to the *B. rapa* plants that were present within the 1 km boundary. That no evidence was found within the 1 km boundary would indicate that the risk of transfer in areas beyond the 1 km boundary is unlikely, although research to test the potential for gene transfer over large distances would be useful.