

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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Final Report

Comissioned by: Office of the Gene Technology Regulator

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Table of contents

| | |
|--|---|
| Introduction | 3 |
| Materials and Methods | 3 |
| General Procedures | 3 |
| Trial sites | 3 |
| Mapping of B. rapa | 4 |
| Herbicide applications | 4 |
| Trial Schedule | 4 |
| Colebrook site | 5 |
| Mapping of plants | 5 |
| Assessment of herbicide damage | 5 |
| Cambridge site | 6 |
| Mapping of plants | 6 |
| Assessment of herbicide damage | 7 |
| Conclusion | 7 |

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 were 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 etrex) 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 m² 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 <i>B. rapa</i> at the Colebrook site and spraying with Roundup |
| 16/07/02 | Mapping of <i>B. rapa</i> at the Cambridge site |
| 22/07/02 | Spraying of <i>B. rapa</i> at the Cambridge site |
| 05/08/02 | Assessing <i>B. rapa</i> at the Colebrook and Cambridge sites and re-spraying |

12/08/02 surviving plants at the Cambridge site
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 diameter rosettes |
| 050 | 535959E : 5261250N | 1 flowering plant |
| 051 | 535953E : 5261241N | 3 plants 10 cm diameter 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 diameter 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 diameter 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.