rate slow enough to reassure the public that we were moving carefully. Consumer confusion caused by a lack of independent investigation has become a lack of confidence in the regulatory system for food safety. The only way to address this is to encourage independent food safety testing of the products before they are put on the market. No one would accept the results of a quality assurance audit if it was undertaken by those with a vested interest in the results and so will it be with the food safety testing of GM derived products.

The harder you push for acceptance the more resistance you will meet, the concerns of farmers and consumers must be acknowledged and respected. Now is not the time for million dollar advertisement or 'education' campaigns about the benefits of biotechnology it is the time for honest, transparent and accountable debate.

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The myths of gene transfer – a canola case study

P.A. Salisbury[A,B]
[A] Institute of Land and Food Resources, University of Melbourne, Parkville, Victoria 3052, Australia.
[B] Agriculture Victoria, Victorian Institute for Dryland Agriculture, Private Bag 260, Horsham, Victoria 3401, Australia.

Summary
Canola (*Brassica napus*) is not a significant weed in managed ecosystems, nor is it invasive of natural ecosystems. Canola incorporating herbicide tolerance (HT) genes has no altered weed or invasiveness potential. The novel trait confers no competitive advantage unless plants are challenged with the specific herbicide. Multiple HT canola volunteers are no more difficult to control in following crops than conventional or single HT canola. They are susceptible to a range of conventional herbicides representing a number of different herbicide groups. There are significant barriers to the introgression of HT genes into the genome of weedy species. However, should introgression occur, any HT weeds, as with HT volunteers, would be controlled using other available herbicides. Enhanced management practices will be required to minimize HT gene flow, either through pollen transfer or seed movement, to non-HT canola, to other HT canola types and to weedy species.

Introduction
Genetically modified (GM) canola (*Brassica napus*) offers considerable benefits to the Australian industry, including potentially higher yields, a healthier and broader product range and renewable oil sources. The first GM canola products to be available in Australia commercially will be herbicide tolerant (HT) cultivars, which bring major benefits in terms of enhanced weed control and higher yields. However, there are also a number of potential concerns with the development of HT canola cultivars, including the potential that addition of the herbicide tolerance gene will make canola a weed of agriculture and invasive of natural habitats. Further, there are concerns of potential gene flow from HT canola to other canola crops and to wild relatives, whose offspring may become more weedy or invasive. This report evaluates these concerns and presents some management suggestions for HT canola.

Potential weediness of HT canola
Canola (*B. napus*) is not a significant weed in managed ecosystems, nor is it recorded as being invasive of natural ecosystems (AAFC 1994). Results from Canada and the UK have shown that the incorporation of a HT gene into *B. napus* has not altered its weediness or invasive potential (AAFC 1995a,b,c,d, 1996a,b, Rasche and Gadsby 1997, PBO 1998, Norris *et al.* 1999). Like non-HT canola, HT canola is not a significant weed in managed ecosystems, nor is it invasive of natural ecosystems.

Studies of reproductive and survival characteristics of HT canola, incorporating vegetative vigour, overwintering capacity, flowering period, time to maturity, seed production and dormancy, showed that the HT canola values fell within the normal range of expression of characters in unmodified *B. napus*. This has been shown for all novel HT types, including different transformants with glyphosphate tolerance (AAFC 1995b, 1996a), glufosinate-ammonium tolerance, including where the HT gene has been combined with the hybrid system (AAFC 1995a,d, 1996b), bromoxynil tolerance (PBO 1998) and the non-GM imidazolinone tolerance (AAFC 1995c).

The number of HT volunteers in the year following GMO trials varies widely, and is influenced by trial size, harvesting conditions and environmental conditions (Norris *et al.* 1999). The numbers of HT volunteers in the year following trials are comparable to, or less than, unmodified *B. napus* in both Canadian and UK trials (Crawley *et al.* 1993, Booth *et al.* 1996, Hails *et al.* 1997, Rasche and Gadsby 1997, Sweet *et al.* 1997, Norris *et al.* 1999). HT volunteers do not show increased numbers or fitness relative to conventional volunteers (Messean 1997, Norris *et al.* 1999, Sweet and Shepperson 1998, Sweet *et al.* 1999a,b). GM HT canola did not lead to increased problems of volunteer management in subsequent crops (Norris *et al.* 1999).

Monitoring results from unmanaged areas adjacent to fields and along transportation corridors in Canada indicated that the frequency of HT volunteers is equal to traditional volunteers. Both are equally likely to appear by the roadside if seed falls from trucks or farming equipment (Rasche and Gadsby 1997, MacDonald personal communication). Evidence from Canada (MacDonald personal communication) indicates that roadside populations of canola only survive if they

are regularly replenished with new seed. HT volunteers in unmanaged areas or along roadsides can be controlled by appropriate herbicides or herbicide mixtures or use of other means of management (e.g. slashing).

Agronomic characteristics, stress adaptation (other than tolerance to specific herbicides) and qualitative and quantitative composition of HT types are also within the normal range of values displayed by conventional cultivars, confirming that plant pest potential has not been altered (AAFC 1995a,b,c,d, 1996a,b, PBO 1998). Likewise, seed morphology and average seed weight of the HT types did not change relative to their non-HT counterparts, indicating that seed dispersal potential had not altered (AAFC 1995a,b,c,d, 1996a,b, PBO 1998). It is evident that the incorporation of HT genes has not altered weed or invasiveness potential of canola.

Gene flow

Gene flow (dispersal) can occur both by pollen transfer and by seed movement (Messean 1997, Champolivier *et al.* 1999a,b, Rieger *et al.* 1999).

Pollen transfer

Canola pollen is transferred by wind and by insects, especially honey bees (Williams *et al.* 1986, 1987, Scheffler *et al.* 1993, Paul *et al.* 1995, Timmons *et al.* 1995, Thompson *et al.* 1999). The vast majority of pollen travels less than 10 m (Scheffler *et al.* 1993), but pollen can disperse over much longer distances. Recorded extremes are 1.5 km for wind movement (Timmons *et al.* 1995) and 4 km with insects (Thompson *et al.* 1999). Over 82% of pollen grains recorded more than 100 m from their source were present as single grains, rather than clumps (Thompson *et al.* 1999).

There are very significant effects of regional distribution and environmental and experimental conditions on the amount of pollen movement (Gliddon 1999, Thompson *et al.* 1999). For example, unfavourable (cold, wet) weather during flowering can reduce insect activity in canola and thereby reduce potential gene dispersal.

Results suggested that insects play an important role in pollination, especially over longer distances (Ramsay *et al.* 1999, Thompson *et al.* 1999). With honey bees, Ramsay *et al.* (1999) detected some GM HT pollen in largely non-GM pollen loads. The non-GM pollen source was 500 m from the hive, while the GM pollen source was 800 m away. The results indicate either switching between crops, long persistence of pollen grains on bees, or mixing within the hive. Ramsay *et al.* (1999) found that most honey bee colonies forage up to 2 km from their hive, indicating potential for pollen transfer and fertilization up to 4 km away.

One major method for detecting movement of pollen grains has been using bait plants (often male-sterile or emasculated) to detect outcrossing (often using a HT marker gene). Outcrossing tends to decrease with increasing distance from pollen source (Scheffler *et al.* 1993, 1995, Simpson *et al.* 1999). In a range of studies differing in location, environmental conditions and trial designs, outcrossing has been detected at low levels at up to 47 m (Scheffler *et al.* 1993), 100 m (Manasse and Kareiva 1991, Downey 1999b), 366 m (Stringam and Downey 1982) and 400 m (Scheffler *et al.* 1995) using fertile recipient plants. With male-sterile or emasculated recipient plants, outcrossing has been detected at 400 m (Simpson *et al.* 1999), 1.5 km (Timmons *et al.* 1995) and 4 km (Thompson *et al.* 1999).

Isolation distances of up to 500 m are generally considered sufficient to prevent outcrossing and maintain seed purity (Scheffler *et al.* 1995, Hancock *et al.* 1996).

Seed movement

Seed movement can cause gene flow over time and space. Gene flow over time occurs when seed remains in the field and volunteers in future years. *B. napus* has no real dormancy (Buzza 1979), with most volunteers germinating within two years.

In Australian HT canola trials, the vast majority of HT volunteers have germinated in the first year following the HT canola, with relatively few volunteers the following year. Volunteers were only seen in the third year under exceptional circumstances, such as two near-drought years following a HT trial. In the UK, the number of volunteers tended to be lower in the year following the GM HT canola, and more prevalent in the second crop post-GMO (Norris *et al.* 1999). Volunteers were persisting in soil for up to three years post-GMO at some sites (Norris *et al.* 1999).

However, canola can occasionally survive in the soil for several years due to environmentally induced secondary dormancy (Lutman 1993, Lutman and Lopez-Granados 1998, Lopez-Granados and Lutman 1998). Post-harvest burial, when the soils are dry, provides conditions for secondary dormancy to develop.

Gene flow over space occurs when seed is moved around the farm via harvesting and cleaning equipment and beyond the farm via leakage during transport (Messean 1997, Champolivier *et al.* 1999a,b, Orson and Oldfield 1999, Rieger *et al.* 1999). Good seed handling and management procedures are essential to minimize this means of gene flow.

Outcrossing to other canola (*B. napus*)

There are no sexual barriers to cross-pollination with other *B. napus* crops, so crossing between different *B. napus* crops will occur, with HT genes transferring to close neighbouring crops and fenceline plants. Multiple HT canola will develop if crops are sown sufficiently close together. This multiple HT canola can be readily controlled with a range of herbicides. Levels of outcrossing tend to decrease with increasing distance from the source (Scheffler *et al.* 1993, 1995, Simpson *et al.* 1999).

Competition between 'foreign *B. napus* pollen' and 'selfed *B. napus* pollen' is important in outcrossing. *B. napus* crops produce 5×10^{12} pollen grains per hectare (Chévre *et al.* 1999a). Any 'foreign' pollen coming from outside is potentially competing with this pollen.

Where male-sterile *B. napus* plants are used to measure outcrossing, rather than fertile *B. napus* plants, much higher levels of outcrossing are detected. For example, a UK study (Simpson *et al.* 1999) detected cross-pollination at 400 m from a large GM HT field when using male sterile (non-HT) bait plants, while no cross-pollination was detected on fertile non-HT plants 120 m from the HT source. It is evident, therefore, that the use of male sterile plants to detect outcrossing can produce misleading results.

Forage rape (*B. napus*) is mainly grown in the higher rainfall areas of south western Victoria, South Australia and Tasmania. Gene flow from HT canola (*B. napus*) to *B. napus* forage rape is possible. However, forage rape crops rarely flower and are usually consumed by animals well before seed development. The likelihood of HT gene transfer to forage rape is therefore very low.

Outcrossing to organic canola

Current requirements for organic canola in Australia and elsewhere require complete freedom from GMO's. However, no system of field production for canola can guarantee 100% purity or complete freedom from GM pollen (Moyes and Dale 1999). To ensure successful co-existence of organic and GM canola crops, organic growers need to accept similar standards of purity to those currently used for canola seed production crops worldwide, allowing for example, a threshold of up to 1% off-types (Moyes and Dale 1999). Such thresholds are currently being considered by organic growers in Europe.

When appropriate isolation distances are used, no contamination above allowable thresholds has been reported (Moyes and Dale 1999). Reliable tests are required for detection of low levels of GMO contamination.

Outcrossing to *B. rapa*

Brassica napus (AACC) and *B. rapa* (AA) have a common set of chromosomes, making interspecific outcrossing more likely.

Occurrences of interspecific hybrids in the field have been reported in Canada, New Zealand, UK and Denmark. Frequency of hybrids depends on parental genotypes, experimental design, population size etc. (Palmer 1962, Bing *et al.* 1991, Jorgensen and Andersen 1994, Jorgensen *et al.* 1996, 1998, Landbo *et al.* 1996, Hauser *et al.* 1998, Scott and Wilkinson 1998, Jorgensen 1999).

Where hybridization occurs with *B. napus* as the female, hybrid seed will be harvested and removed along with canola. However, generally more hybrids are found on *B. rapa* (Jorgensen and Andersen 1994, Hauser *et al.* 1997, Jorgensen *et al.* 1998).

A UK study (Scott and Wilkinson 1998) of *B. rapa* populations growing outside of *B. napus* fields found low levels of hybrids (0.4–1.5%) in 7% of *B. rapa* populations, with no other hybrids in the other 93% (Snow and Jorgensen 1999).

Where natural interspecific hybrids have occurred, they have reduced fertility and low seed set (average 2–5 per pod) compared with the parents (Jorgensen and Andersen 1994). When interspecific hybrids are present, spontaneous backcrossing takes place at very low frequency (Hauser *et al.* 1998). Introgression of HT transgenes from *B. napus* to *B. rapa* has occurred (Jorgensen 1999).

However, there is no evidence that the presence of an introgressed HT gene in *B. rapa* has increased its fitness or spread as a weed relative to conventional, non-GM *B. rapa* (Snow and Jorgensen 1999, Sweet *et al.* 1999a).

In Australia, *B. rapa* is not a widespread agricultural weed. Hybrids between *B. napus* and *B. rapa* have not been observed in Australia, except in plant breeders' nurseries (Wratten and Salisbury unpublished data). If introgression of the HT gene occurs, resulting HT *B. rapa* is easily controlled with other herbicides. It is possible that HT *B. rapa* will at some stage be commercially released as a crop for Australia.

Outcrossing to *B. juncea*

Brassica napus (AACC) and *B. juncea* (AABB) have a common set of chromosomes, making interspecific outcrossing more likely. Spontaneous occurrence of interspecific hybrids in the field have been reported in Canada (Bing *et al.* 1991, Frello *et al.* 1995, Jorgensen *et al.* 1998). Interspecific hybrids have reduced fertility (e.g. pollen fertility 0–28%) and low seed set. Where interspecific hybrids are present, backcrossing can take place at very low frequency (Frello *et al.* 1995).

Introgression of HT genes from *B. napus* to *B. juncea* is likely to occur. However, there is no evidence that the presence of an introgressed HT gene in *B. juncea* will increase its fitness or spread as a weed relative to conventional, non-GM *B. juncea.*

There are very small areas of commercial condiment *B. juncea* production in Australia. As a weed, *B. juncea* has a very restricted distribution in Australia. Hybrids between *B. napus* and *B. juncea* have not been observed in Australia, except in plant breeders' nurseries (Wratten and Salisbury unpublished data). If introgression of the HT gene occurs, resulting HT *B. juncea* is easily controlled with other herbicides. It is possible that HT *B. juncea* will at some stage be commercially released as a crop for Australia.

Outcrossing to vegetable *Brassicas*

Gene flow from HT canola (*B. napus*) to *B. napus* vegetables (swedes, rutabaga, Siberian kale) is possible. Likewise, gene flow to *B. rapa* vegetables (e.g. turnip, Chinese cabbage, pak choi) is possible, due to a common set of chromosomes. However, *B. napus* and *B. rapa* vegetables are not recognized as weeds in agricultural environments in Australia. In addition, they are generally harvested prior to flowering.

No hybrids have been reported in the field between *B. napus* and *B. oleracea* vegetables (cauliflower, brussel sprouts, broccoli, several kales, kohlrabi etc.). Again, *B. oleracea* vegetable crops are generally harvested prior to flowering and seed development, unless being used as a seed production crop.

Outcrossing to weedy species

In evaluating the likelihood of outcrossing and potential gene transfer (introgression) to weedy species, there are a number of pre- and post-fertilization issues to consider which will influence the success of gene transfer (Scheffler and Dale 1994, Salisbury and Wratten 1997). Pre-fertilization considerations include physical proximity, synchrony of flowering, breeding system, floral characteristics and competitiveness of pollen. Post-fertilization considerations include sexual compatibility, hybrid viability, fertility of progeny and successful introgression.

The occurrence of hybrids is an intermediate step only, as the HT gene in a hybrid remains on a *B. napus* chromosome. Gene transfer cannot be said to have taken place until the HT gene has been incorporated (introgressed) into the chromosomes of the weedy species through recombination and backcrossing.

Hybrids between *B. napus* and 10 Australian weedy *Brassicaceae* species have been reported following hand pollination and the use of sophisticated embryo rescue methods. This data has been reviewed by Salisbury (1991), Scheffler and Dale (1994), Salisbury and Wratten (1997) and Rieger *et al.* (1999a). However, it is important to note that hybridization data following hand pollination and the use of sophisticated rescue methods gives no measure of likelihood of successful hybridization in nature (Scheffler and Dale 1994).

Naturally occurring hybrids in the field between *B. napus* and weedy species have been reported for three species occurring in Australia: *Raphanus raphanistrum* (wild radish), *Hirschfeldia incana* (Buchan weed) and *Sinapis arvensis* (charlock). The potential for outcrossing and gene introgression in these three species will be examined in more detail.

Outcrossing to *Raphanus raphanistrum*

Naturally occurring hybrids between HT *B. napus* and *R. raphanistrum* in the field have been reported at very low frequencies (Darmency *et al.* 1995). If male sterile *B. napus* is used, the frequency of HT hybrids increases (Eber *et al.* 1994, Darmency *et al.* 1995, Chévre *et al.* 1996). There are significant differences between different male-sterile lines in their effectiveness in producing hybrids (Baranger *et al.* 1995).

The frequency of HT hybrids is lower when wild radish is the female (Darmency *et al.* 1998). Based on results from a range of experimental conditions, sites and years, Chévre *et al.* (1999a,b) estimated that with wild radish as the female, the expected frequency of HT hybrids in wild radish seed was $3 \times 10^{-5} - 10^{-7}$ hybrids. With *B. napus* as the female, the expected frequency of HT hybrids was $5 \times 10^{-4} - 2 \times 10^{-5}$ in small *B. napus* seeds. Such hybrids are sterile (Pinder *et al.* 1999).

When grown in mixtures with wild radish, each HT hybrid produces less than one backcross seed per plant (Darmency *et al.* 1995). The fertility improved in subsequent backcross generations with wild radish, however with each backcross, the percentage of HT plants decreased (Chévre *et al.* 1997, 1998). None of the HT tolerant plants in the BC_3–BC_5 had the same number of chromosomes as wild radish (Chévre *et al.* 1997, 1998, 1999a,b). Downey (1999a,b) noted that French researchers had found significant barriers to the introgression of *B. napus* genes into the wild radish genome. Introgression of the HT gene into the wild radish genome has not occurred.

Outcrossing to *Hirschfeldia incana*

Hybrids between HT *B. napus* and *Hirschfeldia incana* in the field were initially reported at very low frequencies using male sterile *B. napus* (Lefol 1993, Eber *et al.* 1994, Chévre *et al.* 1996). With male sterile *B. napus,* 1.9 hybrid seed set per 100 male-sterile *B. napus* flowers, while with *H. incana* as the female, there was extremely low frequency of hybrid production (Eber *et al.* 1994, Chévre *et al.* 1996).

The reproductive fitness of the hybrids was very low, with each producing

0.1–0.2 viable seeds per plant (Lefol *et al.* 1996a,b). Chévre *et al.* (1999a) estimated the fitness of hybrids at 10^{-6} relative to the parents and suggested that the hybrids should not be a troublesome problem if good weed management practices were used. Downey (1999a,b) reported that the French researchers have found significant barriers to the introgression of HT genes into the *H. incana* genome.

Outcrossing to *Sinapis arvensis*

Successful hybrids between *B. napus* and *Sinapis arvensis* were only detected when male sterile HT *B. napus* was used as the female, with six hybrid seed from 50 000 *B. napus* plants (Lefol *et al.* 1996b). With *S. arvensis* as the female, no hybrids were detected among 2.9 million seeds (Lefol *et al.* 1996b). All hybrids were sterile. There is general agreement that no gene flow (introgression) will occur between *B. napus* and *S. arvensis* (Downey 1999a,b).

Outcrossing to other weedy species

No natural hybrids have occurred with other Australian weedy species in the Tribe Brassiceae e.g. *Brassica tournefortii, B. oxyrrhina, Diplotaxis muralis, D. tenuifolia, Rapistrum rugosum*.

No hybrids (even with hand pollination and embryo rescue techniques) have been obtained with weedy crucifer species in other tribes, e.g. *Myagrum perfoliatum, Capsella bursapastoris, Sisymbrium* spp., *Cardaria draba* (Salisbury 1991).

Overall

If HT tolerant weedy individuals ever arose through interspecific or intergeneric hybridization, followed by backcrossing and introgression, the novel trait would confer no competitive advantage unless plants were challenged with the specific herbicide (Downey 1999a,b). Any hybrids, as with HT volunteers, would be controlled using other available herbicides (AAFC 1995 a,b,c,d, 1996 a,b, PBO 1998). Hybrids, if they developed, could potentially result in the loss of a specific herbicide as a tool to control this species. This can however be avoided by the use of sound management practices.

AAFC (1995 a,b,c,d, 1996 a,b) and PBO (1998) concluded that while HT gene flow to canola weedy relatives is possible, it would not result in increased weediness or invasiveness of these relatives.

Management of HT canola

Enhanced management practices will be required to minimize HT gene flow (either through pollen transfer or seed movement) to non-HT canola, to other HT canola types and to weedy species. Some potential practices which could be incorporated into a management plan for HT canola are listed below.

- Adopt management practices to minimize the number of volunteers left in the paddock following harvest.
- Delay cultivation to discourage burial of seed after harvest, and thereby prevent development of secondary dormancy. Otherwise, seed may remain in the field to germinate and become volunteer weeds in future years (Pekrun *et al.* 1997, 1998).
- Control all volunteers in subsequent crops, using an appropriate herbicide or herbicide mixture.
- Choose herbicide or herbicide mixture for conservation tillage or for in-crop volunteer control which acknowledges other potential HT crops grown near previous HT crop.
- Utilize the wide range of herbicides available for HT canola control.
- Control volunteers along fencelines and roadsides and around sheds and silos.
- Maintain isolation distances between different HT types within a property and, where possible, between properties. Growers must not mix HT types within a paddock.
- If two different HT types are sown side-by-side on adjacent properties, consideration should be given to a 10 m buffer between HT types, to reduce pollen flow (Pierre and Renard 1999).
- Clean harvesting and cleaning equipment to minimize seed movement.
- Use well sealed trucks to prevent seed loss during transport.
- Growers to keep good paddock records, including seed production lot numbers.
- Preferably use new certified seed each year, regardless of whether HT or non-GM canola.
- If farmer retained seed is used, the seed must not be kept from a HT paddock where a different HT crop was sown nearby.
- Store different HT types separately, if seed is being retained for future sowing.
- Resellers / agronomists should be accredited before being able to sell seed.
- Resellers / agronomists need to advise growers on best management practices for HT canola.
- Consideration should be given to rotation of different HT types within a paddock. This will minimize the likelihood of build up of HT weeds through over use of individual chemicals.

Management of multiple HT canola volunteers

Multiple HT canola volunteers have occurred where several HT types were grown in the one yield trial or in adjacent fields (Champolivier *et al.* 1999a,b, Simpson *et al.* 1999) and where a farmer grew several HT types together (Downey 1999a). Multiple HT canola volunteers are no more difficult to control in following crops than conventional or single HT canola (Norris *et al.* 1999, Orson and Oldfield 1999, Simpson *et al.* 1999). They are susceptible to a range of conventional herbicides used on other crops, representing a number of different herbicide groups.

Multiple HT canola is no more weedy or invasive than single HT or non-HT canola types (Downey 1999a,b). The range of herbicides available for control of multiple HT canola is reduced. However, the choice of an appropriate herbicide for volunteer control will still readily eliminate these types.

It is considered that sound management practices will prevent serious problems from arising with multiple HT volunteers, while at the same time providing growers and processors with improved quality cultivars (Barber 1999).

Management of HT weeds

If any HT hybrids occur between *B. napus* and weedy species, where *B. napus* is the female, the HT hybrid seed will be harvested and removed from the paddock along with the canola seed. The herbicide chosen to remove HT volunteers from the previous canola crop is also likely to remove any remaining HT hybrids. Elimination of canola and weeds along fencelines and roadsides will remove further HT hybrids.

Should introgression occur, HT weeds are no more weedy or invasive than the non-HT weed, except in the presence of the specific herbicide (AAFC 1995 a,b,c,d, 1996 a,b, PBO 1998).

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