

# A spatial assessment of *Brassica napus* gene flow potential to wild and weedy relatives in the Fynbos Biome

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Gene flow between related plant species, and between transgenic and non-transgenic crop varieties, may be considered a form of biological invasion. *Brassica napus* (oilseed rape or canola) and its relatives are well known for intra- and inter-specific gene flow, hybridisation and weediness. Gene flow associated with *B. napus* poses a potential ecological risk in the Fynbos Biome of South Africa, because of the existence of both naturalised (alien, weedy) and native relatives in this region. This risk is particularly pertinent given the proposed use of *B. napus* for biofuel and the potential future introduction of herbicide-tolerant transgenic *B. napus*. Here we quantify the presence and co-occurrence of *B. napus* and its wild and weedy relatives in the Fynbos Biome, as a first step in the ecological risk assessment for this crop. Several alien and at least one native relative of *B. napus* were found to be prevalent in the region, and to be spatially congruent with *B. napus* fields. The first requirement for potential gene flow to occur has thus been met. In addition, a number of these species have elsewhere been found to be reproductively compatible with *B. napus*. Further assessment of the potential ecological risks associated with *B. napus* in South Africa is constrained by uncertainties in the phylogeny of the Brassicaceae, difficulties with morphology-based identification, and poor knowledge of the biology of several of the species involved, particularly under South African conditions.

**Key words:** ecological risk assessment, herbicide resistance, Brassicaceae, hybridisation, species distribution range, biotechnology, transgenic

## Introduction

Gene flow from crops to wild relatives has been associated with the evolution of weediness in seven of the world's 13 most important crops.<sup>1</sup> In addition, of the environmental risks posed by transgenic crops, those associated with the transfer of transgenes are considered to be most important.<sup>2,3</sup> Gene flow between crops and wild relatives is well documented,<sup>1,4</sup> and the possible movement of transgenes from crop plants to wild relatives must thus be considered in (i) the ecological risk assessment for transgenics, (ii) the introduction and development of novel crops, and (iii) agricultural expansion for biofuel production.<sup>2,5,6</sup>

Gene flow from transgenic crops to wild relatives may have a number of negative effects.<sup>3</sup> For instance, transgenic technology has the potential to exacerbate the invasiveness of plant species.<sup>7</sup> Hybridisation with transgenic varieties could increase the fitness of a weed species (by for example conferring traits such as drought tolerance), or compound the effects of existing invasive species.<sup>8–10</sup> The possible long-term ecological effects of such inva-

sion may be considerable.<sup>11–13</sup> Furthermore, gene flow from transgenic plants is difficult to contain.<sup>2</sup> This has been clearly demonstrated by transgene movement in maize,<sup>14,15</sup> rice,<sup>16</sup> creeping bentgrass<sup>17,18</sup> and oilseed rape.<sup>19,20</sup> Indeed, Snow and Morán-Palma<sup>2</sup> suggest that 'if gene flow is possible then it is probable'. Nonetheless, for hybridisation to occur between a crop plant and a wild relative, a number of barriers to gene flow must be overcome.<sup>21</sup> For example, the respective taxa must be geographically proximate, must overlap at least partially in flowering time, must share a pollination mechanism, must show reproductive compatibility, and hybrids must be viable and at least partially fertile.<sup>22</sup>

One of the species of concern is *Brassica napus* L. (Brassicaceae), i.e. canola or oilseed rape.<sup>23–25</sup> *Brassica napus* has a number of characteristics favouring gene flow and a potential increase in weediness. These include the ability of *B. napus* to form volunteer populations, as well as its propensity to become weedy in other cultivated crops.<sup>26</sup> Gulden *et al.*<sup>19</sup> reported *B. napus* seed losses during harvest as 20 times the normal seeding rate. These seeds can remain in the soil seedbank for several years after harvest.<sup>26</sup> *Brassica napus* seed and pollen also have high mobility. For instance, for a number of seed lots containing certified transgenic and non-transgenic *B. napus* seed, 97% had adventitious contamination.<sup>27</sup> Oilseed rape has large pollen dispersal potential and it can outcross and hybridise with wild relatives, such as *Brassica rapa* and *Raphanus raphanistrum*.<sup>20</sup> Indeed, hybridisation between several representatives of the genus *Brassica* and sexually compatible (non-transgenic) wild relatives has been regularly reported.<sup>28</sup>

*Brassica napus* is becoming one of the most important sources of oil and protein in the world.<sup>29</sup> It is currently the fourth most important oilseed and global production continues to increase rapidly.<sup>30</sup> In addition, transgenic, insect-resistant and herbicide-tolerant *B. napus* varieties have been developed and tested in field experiments.<sup>31,32</sup> *Brassica napus* (non-transgenic) was introduced to South Africa fairly recently, with 5 000 ha planted in 1994, and 40 200 ha planted to the crop in 2005/06.<sup>33</sup> It has also been identified as a possible crop for the production of biofuel in South Africa,<sup>34</sup> and the area planted to *B. napus* may thus increase significantly in the future. With a larger area planted to *B. napus* there may well also be more interest in the use of transgenic *B. napus*. Transgenic *B. napus* with glufosinate ammonium tolerance was approved for trial release in South Africa in 2000,<sup>35</sup> although to date it has not received market approval. There has been little attempt to identify the possibility of gene flow from transgenic crops to wild or weedy relatives in the country, despite a constant increase in the number of field trials of a range of transgenic crops that have been approved.<sup>35,36</sup> Although elsewhere there have been several studies of gene flow in *Brassica* species and their wild relatives,<sup>23,37</sup> the likelihood and consequences of gene flow in this system in South Africa have to date not been considered, nor have the consequences of increasing area planted to the crop.<sup>38</sup>

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Here we: (i) quantify the diversity of wild and weedy *B. napus* relatives, and (ii) assess the spatial congruence of their distributions as a basis for understanding the potential for gene flow from commercially-produced *B. napus* to wild and weedy relatives in the Fynbos Biome of the Cape Floristic Region. This is a globally significant centre of biodiversity and endemism that is highly susceptible to plant invasions.<sup>39–41</sup> The Fynbos Biome also encompasses the majority of the area planted to *B. napus* in South Africa. The results reported here will inform ecological risk assessment and regulatory decisions for envisaged biofuel, and potential transgenic, *B. napus* cultivation,<sup>42,43</sup> and will narrow the range of potential taxa for further experimental assessment of reproductive compatibility.

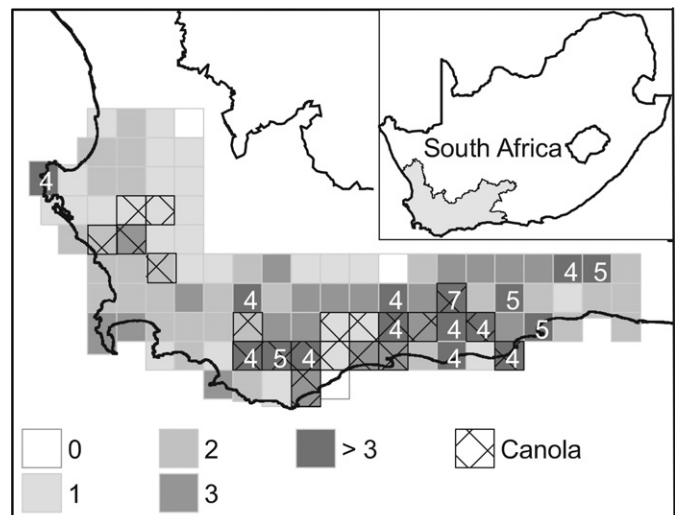
## Methods

The study area was the Fynbos Biome of the Western Cape Province, South Africa (Fig. 1). In this area *B. napus* L. is commercially produced in six municipalities and is locally referred to as canola (and less commonly in South Africa as oilseed rape).

A literature study was conducted to determine which plant taxa may have the potential to hybridise with *B. napus*. All species present in the tribe Brassiceae (containing *B. napus*) and the closely-related tribe Sisymbrieae were considered.<sup>44</sup> We subsequently compared this list of taxa with plant specimens from the Compton Herbarium (South African National Biodiversity Institute (SANBI), Cape Town) and with the Pretoria Computerised Information System (PRECIS) database of the National Herbarium (SANBI, Pretoria) to finalise the target species list. Nomenclature followed Germishuizen and Meyer.<sup>45</sup>

A rapid assessment field survey was conducted to estimate the spatial overlap in ranges between *B. napus* and its wild and weedy relatives in the Fynbos Biome. The rapid assessment approach was adopted based on the assumption that the greatest potential risk for gene flow is with prevalent, wide-ranging species that frequently co-occur with *B. napus*. The study area was divided into quarter-degree square (QDS) grid cells of approximately 23 × 28 km. Of these grids 98 consecutive QDS were selected for the field sampling, encompassing the Fynbos Biome. Field sampling targeted mainly disturbed road verges with agricultural activities in the hinterland. These are common habitats for weedy relatives of *B. napus*, and also represent localities where putative gene flow is most likely to occur (road verges about *B. napus* fields). Road verges were visited between September and October 2008, as close as possible to the centre of each QDS. Based on the herbarium specimens of the Compton and PRECIS collections, the survey period coincided with the main flowering seasons of the target species. At each site we carefully examined the area for approximately ten minutes and recorded the presence of target species. When present, target species were always found within the first two minutes of examination. Specimens were collected for all observations to verify species identification and for future genetic analysis. Additional specimens of target species were collected *ad hoc* while travelling between the QDS centres. A total of 425 records for South Africa and 69 for the Western Cape Province were obtained from the Compton and PRECIS Herbarium databases, and 222 records (different QDS by species combinations) were obtained from the field survey.

The qualitative spatial risk assessment was based on the following factors: (i) Indigenous relatives were considered to present a greater risk than alien relatives, because gene flow with indigenous species potentially represents the greater risk to biodiversity. (ii) Relative prevalence. The higher the relative prevalence of the species in the Western Cape Province and in the field survey, the greater the potential gene flow risk. Here the



**Fig. 1.** Species richness of relatives of *Brassica napus* (canola) in surveyed quarter-degree squares across the Fynbos Biome (Western Cape Province, South Africa). In cells with greater than three relatives the number of species of relatives recorded is shown.

frequency of occurrence of herbarium records of the species, and/or its sampled prevalence in the field survey, was used to calculate relative prevalence. Relative prevalence was scored based on the species representing <5% (score 1), 5–10% (score 2), 11–25% (score 3) and >25% (score 4) of the records in the Western Cape Province or in the field survey. (iii) Spatial overlap. Relatives found to overlap spatially with *B. napus* were considered to present a higher risk for gene flow compared with those with distributions that did not overlap spatially. (iv) Reproductive compatibility. Where available, literature evidence on reproductive compatibility and the formation of hybrids was used to assess hybridisation risk.<sup>46</sup> Within indigenous and alien relative categories, relatives considered to present the greatest risk for gene flow were thus those that had high relative prevalence, overlapped spatially with *B. napus*, and for which there was significant literature evidence of reproductive compatibility. Species were then ranked (1 = highest risk) based on their performance according to these risk factors.

## Results

Based on literature information and the field survey conducted as part of this study, 27 relatives of *B. napus* were identified as occurring in South Africa. These relatives include both alien (mostly naturalised) and indigenous species (Table 1). Because the field survey may be considered a rapid assessment (restricted to road-verge sampling), the list is not necessarily complete. Nonetheless, it is likely to be representative of the most common wild relatives. Further, because some specimens were difficult to identify, a few taxa in the list are not identified to species level. The group is well known for its phenotypic plasticity, and hybrids may share morphological features of parent taxa,<sup>44,47,48</sup> making morphological identification difficult in some cases.

Of the 27 relatives of *B. napus* in South Africa, 13 occur in the Western Cape Province (including the Fynbos Biome), and of these at least seven were sampled in the field survey (including six alien and at least one indigenous species). *Raphanus raphanistrum*, *Brassica tournefortii* and *Sisymbrium capense* were most prevalent in herbaria collections for the Western Cape Province, and *R. raphanistrum*, *Rapistrum rugosum*, *B. tournefortii* and *Hirschfeldia incana* were most prevalent in the field survey (Table 1). *Sisymbrium orientale* and *S. capense* were also moderately

**Table 1.** Species list of *Brassica napus* relatives (*B. napus* included in list in bold) and their proportional occurrences (expressed as a percentage of the total records) in the Compton and PRECIS Herbarium databases nationally (South Africa,  $n = 425$  records), within the Western Cape Province ( $n = 69$ ), and from the field survey (Survey,  $n = 222$ ).

Species	Indigenous (I) or Alien (A) <sup>45</sup>	South Africa	Western Cape Province	Survey
<b>Tribe Brassiceae</b>				
<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>	A	1.4		
<i>Brassica juncea</i> (L.) Czern. & Coss.	A	2.6	4.3	
<i>Brassica napus</i> L.	A			2.7
<i>Brassica nigra</i> (L.)	A	0.2		
<i>Brassica rapa</i> L.	A	2.4	1.4	
<i>Brassica</i> sp.	A	0.2	1.4	
<i>Brassica tournefortii</i> Gouan	A	4.2	11.6	17.6
<i>Eruca sativa</i> Mill.	A	0.9		
<i>Erucastrum arabicum</i> Fisch. & C.A.Mey.	I	0.2		
<i>Erucastrum austroafricanum</i> Al-Shehbaz & S.I.Warwick	I	15.1	1.4	
<i>Erucastrum griquense</i> (N.E.Br.) O.E.Schulz	I	4.5		
<i>Erucastrum strigosum</i> (Thunb.) O.E.Schulz	I	7.1	4.3	
<i>Hirschfeldia incana</i> (L.) Lagr.-Foss.	A	0.7	1.4	13.1
<i>Raphanus raphanistrum</i> L.	A	15.3	27.5	29.3
<i>Raphanus sativus</i> L.	A	0.2		
<i>Raphanus</i> sp.	A	0.2		
<i>Rapistrum rugosum</i> (L.) All.	A	5.4	5.8	19.4
<i>Sinapis alba</i> L.	A	0.5		
<i>Sinapis arvensis</i> L.	A	0.9		
<b>Tribe Sisymbrieae</b>				
<i>Sisymbrium burchellii</i> DC. var. <i>burchellii</i>	I	7.1	4.3	
<i>Sisymbrium capense</i> Thunb.	I	12.7	21.7	7.7
<i>Sisymbrium capense</i> Thunb. x <i>S. turczaninowii</i> Sond.	I	0.2	1.4	
<i>Sisymbrium officinale</i> (L.) Scop.	A	2.1	1.4	0.9
<i>Sisymbrium orientale</i> L.	A	5.6	8.7	8.1
<i>Sisymbrium</i> sp.		1.2	2.9	1.4
<i>Sisymbrium turczaninowii</i> Sond.	I	8.9		
<b>Total</b>		100.0	100.0	100.0

prevalent in the field survey (Table 1).

There is significant overlap between *B. napus* fields and the distribution of several wild and weedy relatives of *B. napus* in the Fynbos Biome (Figs 1 and 2). The maximum number of species per QDS recorded was seven, in the vicinity of Sandkraal (QDS 3321CD) (Fig. 1). Thirty-seven of the sampled QDSs included three or more relatives, and 12 of these high wild relative-species-rich cells overlapped with the presence of *B. napus* (Fig. 1).

There is comparatively little information in the literature on the reproductive compatibility of *B. napus* and wild and weedy relatives found in South Africa (Table 2). However, at least nine naturalised relatives of *B. napus* in South Africa have elsewhere been demonstrated to have some degree of reproductive compatibility with *B. napus* (Table 2). It is important to emphasise that the absence of records of compatibility represents a lack of knowledge and not necessarily the absence of compatibility. No information is available on the potential for reproductive compatibility between *B. napus* and indigenous relatives in South Africa.

The qualitative spatial risk assessment for gene flow potential between *B. napus* and relatives in the Fynbos Biome was based primarily on documented overlap in their spatial distributions, and relative prevalence in herbaria and field survey samples (Fig. 2, Table 3). *Sisymbrium capense* was ranked as the highest priority indigenous species for further examination (Table 3), on the basis that it was most prevalent and spatially congruent with *B. napus*.

Indigenous species in the genus *Erucastrum* should also be considered a priority for further assessment, because elsewhere in the world species in the genus have been shown to be reproductively compatible with *B. napus* (Table 3).

Amongst the naturalised (and weedy) species, *R. rugosum*, *B. tournefortii*, *R. raphanistrum* and *H. incana* are considered to be

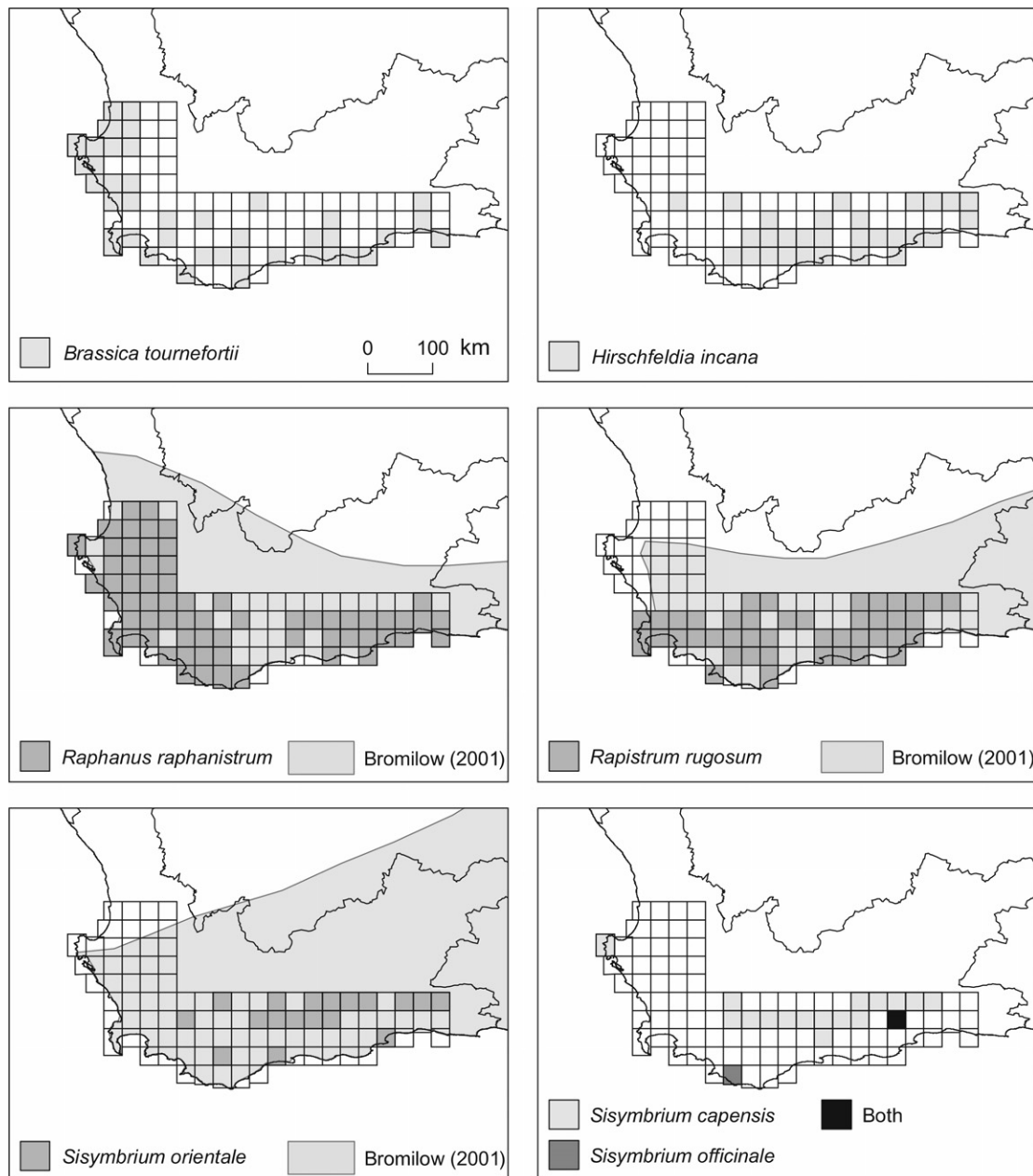
of the highest priority for further gene flow and hybridisation risk assessment (Table 3). All four species are prevalent in the Fynbos Biome, are spatially congruent with *B. napus* and have elsewhere been shown to have some degree of reproductive compatibility with *B. napus* (Table 3).

## Discussion

A general model for assessing the risks associated with gene flow includes three components: (i) factors that affect the likelihood of gene flow, (ii) factors that will affect the likelihood of transgene establishment and proliferation, and (iii) the potential negative consequences of gene flow.<sup>49</sup> One of the first questions included in risk assessment guidelines for transgenic organisms is what factors affect the likelihood of intra- and interspecific gene flow.<sup>50,51</sup> This includes determining if related taxa are present in the region, and if so, their frequencies and distributions.<sup>50</sup> Here we have shown that several taxa, that are closely related to *B. napus*, co-occur with *B. napus* fields in the Fynbos Biome. We have also shown that related taxa are widespread across the Fynbos Biome, and that a number of these are also prevalent (occurring in a high proportion of sites visited). Therefore, based on the co-occurrence and prevalence of relatives of *B. napus* in the Fynbos Biome, at least one hurdle to potential gene flow (i.e. spatial co-occurrence) has been overcome for several taxa.

The spatial overlap in the distribution of relatives is of course in itself insufficient for gene flow and hybridisation to occur. In addition, the majority of attempts to form hybrids reported in the literature have been unsuccessful. One review<sup>46</sup> showed that 47% of reported attempts at hybridisation with *B. napus* were unsuccessful, but that at least one instance of successful hybridisation was found in the remaining species ( $n = 23$ ). The review, however, also found high variability in the success of hybridisation attempts across studies.<sup>46</sup> This suggests that single studies demonstrating unsuccessful hybridisation are insufficient for





**Fig. 2.** Distribution of the seven relatives of *Brassica napus* recorded in the rapid assessment field survey in the Fynbos Biome. Distribution ranges for those species, where available (from Bromilow<sup>70</sup>), are shown as overlays.

reaching conclusions about hybridisation potential in this group of taxa.

Cross pollination between populations and reproductive compatibility, including the fertility, fitness and persistence of hybrids, are also necessary for gene flow to pose a potential risk to biodiversity.<sup>24,52</sup> Although information on the phenology, especially flowering times, of *B. napus* and its relatives in South Africa is patchy, most of these taxa flower in spring and summer, and disjunct flowering seasons are thus unlikely to form a barrier to potential gene flow between them.<sup>53</sup> Again, little is known about the reproductive mechanisms, compatibility and possible fertility of hybrids in these species, particularly under South African conditions. Although in some cases hybrids are known to generally be self-incompatible (e.g. *B. napus* × *B. rapa*) or to have low fitness,<sup>54</sup> the absence of such information on several of these taxa, and under local conditions, is cause for concern. Within South Africa there are five naturalised species in the genus *Brassica*, as well as a number of native and naturalised

representatives from closely-related genera where there may be the potential for gene flow. For example, of the naturalised species in the genus *Brassica* in South Africa, *B. rapa* is one of the diploid parents resulting in tetraploid oilseed rape.<sup>20</sup> Cross-compatibility, without the use of *in vitro* ovule and embryo rescue techniques, has been demonstrated between these two species.<sup>20</sup> Representatives of genera that are closely related to, and form hybrids with members of *Brassica*, include the native genus *Erucastrum*. For instance, *E. gallicum* (non-native) has elsewhere been shown to hybridise with *B. napus*.<sup>37</sup>

Finally, if hybrids or transgenes were to spread and persist as feral populations, potential risks to agricultural production and biodiversity would include, for example, the development of, or an increase in weediness, development of herbicide resistance, invasion of natural habitat, movement of populations into new ecological niches and possible extinction of native species.<sup>11–13,55,56</sup> One example of this concerns *R. raphanistrum*—one of the four naturalised alien species considered to present the greatest gene

**Table 2.** Wild (indigenous and naturalised) taxa that occur in South Africa that have the potential to be reproductively compatible with *Brassica napus*, with example literature evidence.

Taxa	Status	Reference/s
<b>Species with demonstrated compatibility with <i>B. napus</i> elsewhere</b>		
1. <i>Brassica juncea</i>	Naturalised	59, 60
2. <i>Brassica rapa</i>	Naturalised	47, 59, 60
3. <i>Brassica nigra</i>	Naturalised	46
4. <i>Raphanus raphanistrum</i>	Naturalised	37, 47, 59
5. <i>Sinapis arvensis</i>	Naturalised	61
6. <i>Sinapis alba</i>	Naturalised	62
7. <i>Eruca sativa</i>	Naturalised	63
8. <i>Brassica tournefortii</i>	Naturalised	64, 65
9. <i>Hirschfeldia incana</i>	Naturalised	66
10. <i>Rapistrum rugosum</i>	Naturalised	46
11. <i>Raphanus sativus</i> L.	Naturalised	45
<b>Representatives of the genus <i>Brassica</i> with no known records of compatibility with <i>Brassica napus</i></b>		
1. <i>Brassica elongata</i>	Naturalised	45
<b>Species in South Africa with which there are records of compatibility between <i>B. napus</i> and a representative of the same genus</b>		
1. <i>Brassica napus</i> × <i>Erucastrum gallicum</i>	Cross	37
The wild representatives of <i>Erucastrum</i> in South Africa include:		
<i>Erucastrum arabicum</i>	Indigenous	45
<i>Erucastrum griquense</i>	Indigenous	45
<i>Erucastrum strigosum</i>	Indigenous	45
<b>Other genera with wild representatives in South Africa not identified above that are closely related to the genus <i>Brassica</i></b>		
1. <i>Diplotaxis siifolia</i> × <i>Brassica napus</i>	Cross: hybrid obtained through ovary culture	67
2. <i>Diplotaxis tenuifolia</i> × <i>Brassica napus</i>	Cross: <i>in-vitro</i> pollination resulted in hybrid embryo	68
Wild representatives of <i>Diplotaxis</i> in South Africa include		
<i>Diplotaxis muralis</i>	Naturalised	45
3. <i>Eruca sativa</i> × <i>Brassica juncea</i>	Cross: hybrid obtained through protoplast fusion	69
Wild representatives of <i>Eruca</i> in South Africa include <i>Eruca sativa</i>		
	Naturalised	70

flow risk based on the findings of this study. Herbicide resistance has already been demonstrated in this species in the Fynbos Biome.<sup>57</sup> Similarly in Australia, herbicide resistance has evolved in *E. austroafricanum* (a close relative of *B. napus*) (<http://www.weedscience.org/Case/>). Such potential unintended and negative consequences of gene flow between *B. napus* and its relatives in South Africa have previously not been investigated nor, to our knowledge, considered.

The results we show here thus provide a first step in the risk assessment for gene flow between *B. napus* and its relatives in the Fynbos Biome. These results clearly demonstrate that further

attention must be given to ecological risk assessment for *B. napus* in this biodiversity-rich region of South Africa, and we have narrowed the list and identified priority species for further attention. Ecological risk assessment will be particularly critical if transgenic *B. napus* is to be considered for release, or if *B. napus* is to be used for biofuel production (with a likely concomitant increase in area planted<sup>38</sup> in the country). However, significant hurdles to ecological risk assessment for *B. napus* currently include: (i) the remaining uncertainties in the phylogeny and relatedness of taxa in the Brassicaceae,<sup>44</sup> (ii) difficulties with morphology-based identification of these species generally and

**Table 3.** Qualitative spatial risk assessment for potential gene flow and hybridisation between *Brassica napus* and wild and weedy relatives in the Fynbos Biome (Western Cape Province, South Africa). Rank: 1 = highest, 8 = lowest potential risk. Relative prevalence: frequency of occurrence of herbarium records and/or sampled in this survey; 1 = low; 2 = moderate; 3 = high; 4 = very high relative prevalence. Spatial overlap: spatial co-occurrence of taxon and *Brassica napus* in the Fynbos Biome; Not recorded = not found in survey across the Fynbos Biome, but known to be present in the Western Cape Province. Compatibility: known cases of hybridisation with *B. napus*, and between *B. napus* and species in the same genus as that listed (see Table 2).

Rank	Species/taxon	Relative prevalence	Spatial overlap	Species compatible	Compatibility within genus
<b>Indigenous</b>					
1	<i>Sisymbrium capense</i>	3	Yes	Unknown	Unknown
2	<i>Sisymbrium capense</i> × <i>S. turczaninowii</i>	1	Yes	Unknown	Unknown
3	<i>Erucastrum austroafricanum</i>	1	Not recorded	Unknown	Yes
4	<i>Erucastrum strigosum</i>	1	Not recorded	Unknown	Yes
5	<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	1	Not recorded	Unknown	Unknown
<b>Naturalised/alien</b>					
1	<i>Rapistrum rugosum</i>	3	Yes	Yes	
2	<i>Brassica tournefortii</i>	3	Yes	Yes	
3	<i>Raphanus raphanistrum</i>	4	Yes	Yes	
4	<i>Hirschfeldia incana</i>	3	Yes	Yes	
5	<i>Sisymbrium orientale</i>	2	Yes	Unknown	Unknown
6	<i>Brassica juncea</i>	1	Not recorded	Yes	
7	<i>Brassica rapa</i>	1	Not recorded	Yes	
8	<i>Sisymbrium officinale</i>	1	Not recorded	Unknown	Unknown

in South Africa,<sup>58</sup> (iii) the lack of taxonomic expertise, and (iv) poor knowledge of the distribution, phenology, pollination syndromes and reproductive mechanisms, particularly, but not only, of indigenous taxa.

We thank Sarah Davies for initiating the funding opportunity for this research, and for her facilitation thereof and comments on the output. We thank C.A.P.E. and the Centre for Invasion Biology for financial support, A.Y. van Zonneveld for field assistance, and the Compton Herbarium in Cape Town for taxonomic assistance and access to data records.

Received 9 February. Accepted 4 May 2009.

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