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Potential impact of weedy Brassicaceae species on oil and meal quality of oilseed rape (canola) in Australia

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Summary

Brassicaceae weeds are a widespread problem in Australian oilseed rape crops. The weeds not only compete for resources during crop growth, but also have the potential to reduce both oil and meal quality of the harvested crop. This paper investigated oil and meal quality of weedy species from the Brassicaceae family that were collected throughout cropping regions of Australia. Eighty nine lines from 19 species were grown and evaluated in the same environment for their potential to contaminate Australian oilseed rape seed lots. Seed and flowering characteristics of each species were also examined. The glucosinolate concentration of most of the weedy species was greater than 100 µmol g^{-1} of oil-free meal, well above the threshold for meeting oilseed rape quality. Erucic acid content of 18 of the 19 weedy species also exceeded the oilseed rape quality standard of less than 2% erucic acid. This paper highlights the potential of the weedy species to reduce the quality of Australian oilseed rape crops.

Keywords: *Brassica napus*, contamination, glucosinolate, erucic acid

Introduction

Ever since the introduction of oilseed rape (*Brassica napus* var. *oleifera* Del.) into Australia, controlling weed species has been a major issue. The potential impacts of these weeds on yield, oil quality and meal quality of oilseed rape have been a significant challenge. The introduction of herbicide tolerance has significantly enhanced weed control options for growers. However, the development and spread of herbicide resistance in these weeds is a risk for the oilseed rape industry (Lemerle *et al*., 2016). Weeds in the Brassicaceae family pose a particular threat through potential contamination of end product quality.

Until the late 1960s, *B. napus* cultivars had high levels of erucic acid in the oil and high levels of glucosinolates in the meal. The levels of these compounds in the early cultivars were considered nutritionally undesirable, causing palatability and nutritional problems in non-ruminant animals (Vles, 1975; Röbbelen & Thies, 1980; Kjaer, 1981; Fenwick *et al*., 1983; Sauer & Kramer, 1983; Bell, 1984). The first *B. napus* cultivars low in erucic acid and low in glucosinolates was released in Canada in 1974 (Stefansson *et al*., 1961; Stefansson 1983) and in Australia in 1980 (Salisbury *et al*., 2016). Oilseed rape cultivars are now defined as seeds of the genus *Brassica* from which the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromole of specified aliphatic glucosinolates per gram (µmol g^{-1}) of oil-free air-dry solid (Downey, 1990).

In Australia, growers are typically paid on a basic oil content of 42%, with a bonus or penalty if the oil content is above or below the minimum oil content. The remaining seed meal has a protein content ranging from high the 30s to the low 40s (%) (Seberry *et al*., 2016) and is used in stockfeed. Oil content in Australian oilseed rape cultivars has increased by 3% over the last 30 years, with protein content increasing by 1.5% (Potter *et al*., 2016). The fatty acid composition of Australian oilseed rape cultivars has remained relatively constant at around 60% oleic acid, 20% linoleic acid and 10% linolenic acid and nutritionists consider that this fatty acid composition of oilseed rape oil is of extremely high quality (Lin *et al*., 2013).

Weed contamination of oilseed rape crops may negatively impact the oil content of the crop by reducing oilseed rape yield through competition for resources (McMullan *et al*., 1994; Blackshaw *et al*., 2002; Beckie *et al*., 2008). A survey of oilseed rape crops in Australia found that weed incidence ranged from 49 weeds m^{-2} in triazine tolerant crops to 72 weeds m^{-2} in non triazine tolerant cultivars (Lemerle *et al*., 2001). Levels of *Sinapis arvensis* L. (charlock) infestation as high as 50.2% have been observed in plots unsprayed with a triazine class herbicide in South Australia (Potter & Salisbury, 1991). The impact of the weeds on yield and quality of the oilseed rape crops will depend on the degree of weed invasion, weed seed number per plant and the composition of the weed species (Davis *et al*., 1999). Physical factors, such as seed size and shape, will also influence the chances of detecting and separating particular weed seeds from oilseed rape seed and therefore influence the level of weed contamination of the processed oilseed rape grain.

Brassicaceae weeds have the potential to contaminate oilseed rape oil and meal such that it does not meet oilseed rape quality standards (Salisbury, 1991). There are very few comprehensive reports on the seed glucosinolate and oil content of the weedy Brassicaceae species found in Australia. Studies in other countries on a limited number of weedy Brassicaceae species indicate the Brassicaceae weeds are high in antinutritional compounds both in the oil and meal (Hasapis *et al.*, 1981; Horn & Vaughan, 1983). The Brassicaceae weed seeds may directly reduce oil content if they are not separated from the oilseed rape seed (McMullan *et al*., 1994). In addition, if oilseed rape seed lot admixtures with Brassicaceae weeds are not sufficiently cleaned prior to crushing the resulting oil content and oil and meal quality could be reduced, particularly if the oil and meal characteristics of the weeds differ

from the accepted oilseed rape quality standard.

Given the importance of meeting oilseed rape quality standards, there is a need to evaluate the potential impact of weeds on oil content, oil quality and meal quality in Australia. The aim of this study was to evaluate physical characteristics, oil and protein content, fatty acid composition and glucosinolate concentration of Australian populations of Brassicaceae weeds, to determine which commonly found weedy species have the greatest potential to negatively impact the oil and glucosinolate concentration of oilseed rape crops. This report is the first time this number of weedy Brassicaceae species has been compared together in the same environment. It provides critically valuable information to assist in the development of weed management plans in oilseed rape cropping regions of Australia.

Materials and Methods

Plant material

Eighty nine lines, comprising 19 weedy species from the Brassicaceae family were used in the experiments (Table 1). The lines of each species were wild populations, each collected from different cropping regions across Australia and multiplied in uniform glasshouse conditions at Horsham, Victoria (Salisbury, 1991). For the experiments, seed was germinated in gibberellic acid solution (2.5×10^{-6} M) for 5 to 8 days and 5–10 seedlings were transplanted into 20 cm diameter pots in a bird-proof cage in a four replicate, randomised block design. Sowing was timed to match the typical autumn sowing time of the region for oilseed rape (May). The potting mix contained 6:1.5:1.5:1 by volume composted pine bark:lignin peat:quartz grit:fine washed sand, with a fertiliser mix (Appendix 1). The pH of the mixture was adjusted to 6.5 by the addition of lime. Seedling numbers were thinned to four per pot once plants were established. Pots were watered two to three times daily using an automatic watering system.

Table 1 near here

Analyses

Flowering time ranged from September to November, with days to flowering recorded when two of the four plants in a pot had at least one flower. Seed harvested from the four replicates of each of the 89 lines was bulked to provide one sample of each line for seed analyses. Thousand

seed weight was measured by taking samples of 100 seeds from each replicate, drying at 38°C for two weeks to minimise moisture variation, weighing to four decimal places and converting to 1000 seed weight. For species with indehiscent pods, where seeds had to be dissected from the pods, 50-seed samples were used. To measure seed number per pod, five pods were taken from the main stem of all four plants in the fourth replicate and the number of seed in each pod was counted.

Visual shattering resistance assessments were made at maturity on the following 0 to 4 scale:

0 − Very Susceptible (valves re gularly fell off mature pods, pods shattered very readily on impact)

1 – Susceptible (valves did not fall of, pods shattered readily on impact)

2 – Moderate Resistance (pods shattered less readily on impact)

3 – Good Resistance (pods were hard to shatter)

4 – Indehiscent (pods did not shatter, sometimes whole pods or beaks broke off or pod broke into segments)

Oil and protein content were determined using the American Oil Chemists Society (AOCS) methods for determining oil and protein content in oilseeds. Oil content is reported as a percentage in whole seed on a moisture-free basis and protein content is reported as a percentage in oil-free meal. Fatty acid composition was analysed using gas chromatography, with results reported as a percentage of the total fatty acids. Total glucosinolate concentration was determined by the method AOF 4-1.22 (AOF 2007). Glucosinolates are reported as µmol glucosinolates gram^{-1} of oil-free meal on an air-dry basis.

Analyses of variance were carried out using the Genstat statistical package and means and standard error are presented. Linear regression relationships between glucosinolate or erucic acid concentrations of a canola-weed admixture and percent weed contamination (PWC) (Davis *et al*., 1999) were determined by taking into account the observed mean value of glucosinolate and erucic acid concentrations of contaminated seed samples and the mean values of uncontaminated seeds of Australian canola (i.e. 16 µmol g^{-1} of glucosinolate and 0% of erucic acid) reported by Seberry *et al*. (2016). The relationships were then used to estimate the minimum percent contamination by each weed species needed to increase the glucosinolate and the erucic acid concentration of a standard canola seed lot above the canola quality standard (i.e. <30 µmol g^{-1} of glucosinolate and <2% of erucic acid).

Results

Oil, protein and glucosinolate content

Oil content of the weedy species (% in whole seed) ranged from 17.1% to 48.1% (Table 2). Most species had lower mean oil content than the Australian average for oilseed rape of 42% (Seberry *et al*., 2016). Protein content for the weedy species varied from 22.6% to 33.4% in oil-free meal (Table 2).

The glucosinolate concentrations of the weedy species ranged from 44–218 µmol/g in oil-free meal (Table 2), with most species having greater than 100 µmol g^{-1}. The highest glucosinolate concentrations were in the *S. arvensis* lines. *Capsella bursa-pastoris* (L.) Medik, *Camelina sativa* (L.) Crantz and *Sisymbrium irio* L. had relatively lower glucosinolate concentrations than the other weedy species, although they were still greater than the threshold for oilseed rape quality. The within species variability for glucosinolate content was most prominent in *Rapistrum rugosum* (L.) All. and *Raphanus raphanistrum* L..

The simple linear regression model indicated that 7.8% to 50% contamination of oilseed rape seed lots with the range of glucosinolate concentrations observed in the weed species would be required to exceed the glucosinolate threshold for meeting the oilseed rape quality standard (Table 3). This is based on the average glucosinolate concentration in Australian oilseed rape of 16 µmol glucosinolates g^{-1} in oil-free meal in 2015 (Seberry *et al.*, 2016).

Table 2 and 3 near here

Fatty acid profiles

The main fatty acids present in the Australian Brassicaceae weeds included palmitic (C16:0), oleic (C18:0), linoleic (C18:2), linolenic (C18:3), eicosenoic (C20:1) and erucic (C22:1) acids (Table 4), with lesser amounts of other fatty acids. The within species variability for fatty acid composition was much less than the between species variability (Table 4).

Table 4 near here

Oleic acid levels in the weed species were well below the Australian average for oilseed

rape of 64.3% in 2015 (Seberry *et al.*, 2016). The highest oleic acid content in the weeds was only 20.8% in *R. raphanistrum*. In contrast, the weed species had higher levels of linolenic acid than oilseed rape. *Conringia orientalis* (L.) Dumort was the only weed species with less than 10% linolenic acid.

Mean erucic acid content of the weed species ranged from 0.7% to 48.5% (Table 4). Five species, all from the Tribe *Brassiceae*, had greater than 40% erucic acid. Fifteen of the weed species had greater than 20% erucic acid, the exceptions were *C. bursa-pastoris* (0.7%) and *C. sativa* (2.6%) with very low amounts of erucic acid.

Based on the simple linear regression model, less than 10% contamination of oilseed rape seed lots with most of the weed species would result in the seed lot exceeding the threshold 2% level of erucic acid if the weed seed was crushed with the oilseed rape seed (Table 5).

Table 5 near here

Seed and flowering characteristics

Thousand seed weight of the Brassicaceae weedy species ranged from 0.078 g to 6.343 g (Table 6). *Sinapis arvensis, C. orientalis, Myagrum perfoliatum* L. and *R. raphanistrum* had seed similar in size to oilseed rape seed, which has a typical thousand seed weight of 2.9–3.6 g (Zhang *et al*., 2011). Days to flowering of the weedy Brassicaceae species ranged from 80 to 189 days. Many of the lines overlapped the typical number of days to first flower observed in oilseed rape, which ranges from 91 to 130 days depending on cultivar and sowing time (Hocking & Stapper, 2001). Pod shatter resistance of the weedy species ranged from very susceptible to indehiscent (Table 6). The weed species with smaller seed weight were in general more susceptible to pod shattering than the species with larger seed weight, although there were some exceptions, such as *S. arvensis* (Table 6).

Table 6 near here

Discussion

Oil and protein content

Fourteen of the nineteen weedy species had oil content less than 40%. *Carrichtera annua* (L.) DC had the lowest oil content with 17.1%. Protein content of all the weedy species was over 20%, with four lines over 30%. There were several major differences between the oil and protein

content observed in weed species in this study compared with other studies. A major part of this variation is likely to be explained by the inverse relationship between oil and protein in the seed, such that under certain environmental conditions oil is laid down at the expense of protein, and vice versa. For example, in this study, the oil content of *C. sativa* was approximately 8% higher and the protein 8% lower than values reported by Mikolajczak *et al*. (1961) and Earle and Jones (1962). As protein contents were not measured in some comparative studies, such comparisons could not always be made.

The oil content of *R. rugosum* was similar to that reported by Miller *et al*. (1965), but approximately 35% more than Kumar and Tsunoda (1978). Such a discrepancy may be due to different cultivation procedures or pod pre-treatment. Other species where major differences in oil content occurred compared with previous studies were *C. orientalis* (17% higher than in Kumar & Tsunoda, 1978), *Hirschfeldia incana* (L.) Lagr-Fossat (12–17% higher than Kumar & Tsunoda, 1978), *S. irio* (17–22% higher than Mikolajczak *et al*., 1961) and *R. raphanistrum* (10–17% higher than Jones & Earle, 1966). However, the extremely small seed size quoted by Jones and Earle (1966) for . *raphanistrum* would suggest that they may have incorrectly identified the species.

While oil contents in this study tended to be slightly higher than those in previous studies, the oil content of *Brassica oxyrrhina* Coss. was around 10% lower than an earlier Australian report (Quinlivan & Devitt, 1972). Environmental conditions can have a major influence on oil and protein content, with cooler temperatures and better rainfall during the growing season found to enhance oil content in *B. napus* (Jensen *et al.*, 1996; Pritchard *et al.*, 2000).

The oil content of many of the weeds was low compared with the average for oilseed rape of 42% in 2015 (Seberry *et al.*, 2016). Thus, in addition to the negative impact of the weeds on competition for space and resources with the oilseed rape crop, some weed species may have the potential to have a direct negative impact on oil content in admixtures with oilseed rape seed. Beckie *et al*. (2008) found that weed competition reduced the productivity of oilseed rape but did not observe an effect on seed oil and protein content. This appeared to be due to the ability of the cultivated species to out compete the weeds and the presence of many grass weeds, with only two Brassicaceae species weeds present (wild mustard, *Sinapis arvensis* L. and stinkweed, *Thlaspi arvense* L.).

Glucosinolate content

The majority of the Brassicaceae weed species tested had seed glucosinolate contents over 100

μmol g^{-1} of oil-free meal, with *R. rugosum* and *S. arvensis* lines containing over 200 μmol g^{-1} of oil-free meal. Only three species, *C. sativa*, *S. irio* and *C. bursa-pastoris*, had less than 100 μmol $g^{-1}$of oil-free meal.

There have been very few reports comparing the seed glucosinolate contents among a range of wild Brassicaceae species in the same environment. Concentrations in leaf tissue of a number of Brassicaceae species have been measured (Cole, 1976; Greenhalgh & Mitchell, 1976; Mithen *et al.*, 1987), but these are not necessarily indicative of seed concentrations, as glucosinolates in different parts of the plant (leaves, stem, roots and seed) can differ in both type and quantity (Rodman & Louda, 1984; Sang *et al*., 1984). Studies of seed glucosinolate contents (Hasapis *et al*., 1981; Horn & Vaughan, 1983; Schroeder *et al*., 1983) have reported high values (always over 13 mg g^{-1}, sometimes over 100 mg/g) with one exception, 0.55 mg g^{-1} in *Alyssum chondrogynum* (Hasapis *et al.*, 1981). The present study confirmed the high seed glucosinolate values reported by Horn and Vaughan (1983) for *Brassica fruticulosa* Cyr., *B. oxyrrhina* and *Brassica tournefortii* Gouan.

The results showed that, in general, wild Brassicaceae species contain very high glucosinolate levels, which have the potential to reduce the meal quality of the oilseed rape crop if weed control is inadequate. Blackshaw *et al*. (2002) demonstrated that processing oilseed rape seed contaminated with weed seed high in glucosinolates such as *R. raphanistrum* can result in oilseed rape meal with glucosinolate levels above acceptable marketable levels, particularly when weed density is high and weeds emerge at the same time as or shortly after oilseed rape. The linear regression modelling of admixtures with a single weed species showed that 7.78% contamination of an average oilseed rape seed lot with a weed species of 196 μmol g^{-1} of oil-free meal such as *Sinapis arvensis* would be required for the oilseed rape-weed admixture to exceed the glucosinolate limits for oilseed rape quality standards. The quality of the oilseed rape seed lot will influence the effect of the weed on glucosinolate concentration, with oilseed rape of lower than average quality requiring even less weed contamination to exceed the glucosinolate standard. Environmental conditions will influence the glucosinolate concentration in the oilseed rape crop, with several studies showing that *Brassica napus* tends to produce higher levels of glucosinolates in response to stresses like drought and high temperature (Mailer & Cornish 1987; Jensen *et al.*, 1996; Pritchard *et al.*, 2000).

Weed control strategies in Australia currently reduce the risk of weed contamination of oilseed rape crops. However, the development and spread of herbicide resistant weeds is a threat to weed management. There is already evidence of triazine resistance in *R. raphanistrum* (Heap, 2017).

Fatty acid profiles

There were marked differences among the different weedy Brassicaceae species in their fatty acid profiles. However, the range of values did not differ beyond that previously found for the fatty acids of particular interest in the Brassicaceae family. There was little variability in the fatty acid profile among different lines of any given species. A similar lack of intraspecific variability has been reported for some wild Brassicaceae species by Mikolajczak *et al.* (1961) and Appelqvist (1971). However, Appelqvist (1971) reported considerable intraspecific variability for fatty acid profile in *S. arvensis* and *Brassica cretica*. The lack of variability in Australian populations could perhaps reflect a limited number of introductions into Australia. Fatty acids are also very dependent on environmental conditions such as the temperature under which they are grown. The fatty acid profiles of the different species were generally very similar to those reported previously (e.g. Mikolajczak *et al*., 1961; Miller *et al*., 1965; Appelqvist, 1971; Kumar & Tsunoda, 1978). The values in the current study were generally slightly lower for the shorter chain (C16 and C18) fatty acids and slightly higher for the longer chain fatty acids than those reported by Kumar and Tsunoda (1978), perhaps due to environmental differences at ripening (Appelqvist, 1969).

Appelqvist (1971) indicated that the reliability of the data presented in such studies was dependent on two factors, namely the frequent misidentification of many Brassicaceae species, and the likelihood of nursery grown samples being less representative than the original field collected material, given the cross-pollinating nature of some species and the likely embryonic control of fatty acid composition. To be balanced against this, however, is the problem of comparing lines grown in vastly different environments, given the known environmental effects on fatty acid composition (Appelqvist, 1968). For this reason, the lines in this study were all grown in a common environment for evaluation.

Erucic acid content of 18 of the 19 weedy species exceeded oilseed rape quality standards. Levels of erucic acid as high as 48% were observed in Brassicaceae weed species that were reported by Sutherland (1999) to have been particularly important in restricting oilseed rape production prior to the introduction of triazine tolerant varieties in Australia, specifically *R. raphanistrum*, *Sisymbrium orientale* L., *C. bursa-pastoris*, *B. tournefortii*, *R. rugosum*, *S. arvensis* and *M. perfoliatum*. Blackshaw *et al*. (2002) studied the emergence of *R. raphanistrum* with oilseed rape and found that at a density of 16 wild radish plants m^{-2} or higher, if the harvested seed was crushed together with the weed species the erucic acid levels were greater than the acceptable market standards for oilseed rape seed. Regression modelling in this study

also showed that less than 10% admixtures of most weed species with oilseed rape would result in oil that exceeded the erucic acid limit for oilseed rape quality standards.

Seed and flowering characteristics

This is the first time a comprehensive comparison of seed quality characteristics of Australian weedy Brassicaceae species has been combined with an examination of the flowering characteristics of the weedy species under the same environmental conditions. Data on potential weed seed size and seed production per plant can contribute to the development of weed management plans in cropping regions of Australia, particularly in furthering the understanding of weed seed bank reserves and potential impacts on yield. The flowering and seed characteristics also provide a greater understanding of which weedy species are of greatest risk of being present in admixtures with oilseed rape, potentially resulting in the oil not meeting oilseed rape standards if the seeds are crushed together. From a processing perspective, the weed species with very small seed relative to oilseed rape are of less concern as they will almost inevitably not be retained in the harvested and cleaned seed. Weed species with overlapping flowering time to oilseed rape, seed of similar size and shape to oilseed rape and moderately dehiscent pods (that dehisce under harvest pressure) are of greatest risk of remaining mixed with oilseed rape seed during processing, for example *S. arvensis*.

Conclusions

This is the most comprehensive study of Australian Brassicaceae weed species evaluated under the same environmental conditions. Considerable variability for oil and protein content, oil quality and glucosinolate content was evident in the Australian weedy Brassicaceae species. Regression analyses based on erucic acid content and glucosinolate concentration of the weed species indicated that contamination of oilseed rape seed with weeds from the family Brassicaceae risks increasing erucic acid in the oil and glucosinolates in the meal, which would result in a reduction in oilseed rape quality and reduced prices. Examination of seed and flowering characteristics of the weedy species identified the species with the greatest potential to remain in admixtures with oilseed rape. The results highlighted the potential effects of these weeds on oilseed rape seed quality if weed management strategies are inadequate or if herbicide resistance occurs.

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Table 1 Weedy Brassicaceae species examined in the study. Lines of each species were collected from different cropping locations across Australia

Species	Common name	Number of lines

Tribe *Brassiceae:*		
Brassica fruticulosa Cyr.	Twiggy turnip	5
Brassica oxyrrhina Coss.	-	1
Brassica tournefortii Gouan	Wild turnip	8
Carrichtera annua (L.) DC	Ward's weed	3
Conringia orientalis (L.) Dumort	Wild cabbage, Hare's ear, Treacle mustard	2
Diplotaxis muralis (L.) DC	Wall rocket	2
Diplotaxis tenuifolia (L.) DC	Sand rocket, Lincoln weed	7
Diplotaxis tenuisiliqua Del.	-	1
Hirschfeldia incana (L.) Lagr-Fossat	Buchan weed, Hairy Brassica	9
Raphanus raphanistrum L.	Wild radish, Jointed charlock	10
Rapistrum rugosum (L.) All.	Turnip weed, Giant mustard	7
Sinapis arvensis L.	Charlock	5
Tribe *Lepidieae:*		
Capsella bursa-pastoris (L.) Medik.	Shepherd's purse	5
Tribe *Euclidieae:*		
Myagrum perfoliatum L.	Musk weed	2
Tribe *Sisymbrieae:*		
Camelina sativa (L.) Crantz	False flax	1
Sisymbrium erysimoides Desf.	Smooth mustard	2
Sisymbrium irio L.	London rocket	5
Sisymbrium officinale (L.) Scop	Hedge mustard	7
Sisymbrium orientale L.	Indian hedge mustard	7

Table 2 Quality characteristics of weedy Brassicaceae species in comparison with average quality characteristics of Australian oilseed rape*. Mean and standard error are presented for the different lines of each species (except for the three species that were represented by only one line)

Species	Oil content (%)†	Protein content (%)†	Glucosinolates (μmol g^{-1})‡
Brassica fruticulosa	34.5 ± 3.5	23.8 ± 1.6	134 ± 15.5
Brassica oxyrrhina	32.8	24.8	101
Brassica tournefortii	32.2 ± 5.0	22.6 ± 1.0	121 ± 15.0
Carrichtera annua	18.6 ± 1.9	31.3 ± 0.3	162 ± 15.5
Conringia orientalis	32.2 ± 0.1	23.2 ± 0.5	140 ± 11.0
Diplotaxis muralis	35.7 ± 0.8	28.2 ± 0.2	118 ± 7.0
Diplotaxis tenuifolia	42.9 ± 2.1	24.9 ± 1.5	135 ± 11.0
Diplotaxis tenuisiliqua	29.4	28.7	117
Hirschfeldia incana	37.2 ± 2.2	26.1 ± 1.8	157 ± 20.0
Raphanus raphanistrum	45.5 ± 3.3	25.4 ± 2.5	135 ± 43.0
Rapistrum rugosum	41.7 ± 2.0	28.6 ± 2.2	170 ± 46.5
Sinapis arvensis	30.0 ± 3.2	27.5 ± 2.8	196 ± 5.5
Capsella bursa-pastoris	31.5 ± 3.7	26.7 ± 2.5	63 ± 4.0
Myagrum perfoliatum	42.1 ± 1.8	32.6 ± 0.1	183 ± 1.0
Camelina sativa	41.6	24.7	44
Sisymbrium erysimoides	24.3 ± 2.5	33.3 ± 0.1	118 ± 4.0

Sisymbrium irio	35.8 ± 2.6	31.3 ± 1.6	67 ± 8.0
Sisymbrium officinale	26.5 ± 3.1	28.5 ± 0.9	95 ± 7.5
Sisymbrium orientale	32.9 ± 1.2	28.4 ± 1.3	94 ± 10.5

*Average quality characteristics of Australian oilseed rape are 42.0% oil, 39.9% protein and 6 μmol g^{-1} in whole seed (16 μmol g^{-1} in oil free meal) based on the annual Australian Oilseeds Federation survey of crops (Seberry *et al.*, 2016).

†Oil and protein content (%) presented on a moisture-free basis.

‡Glucosinolate content (μmol g^{-1}) presented on an oil-free meal, air-dry basis.

Table 3 Linear relationships between glucosinolate concentration of a canola-weed admixture and percent weed contamination (PWC) for each weed species and the minimum PWC required to increase the glucosinolate concentration of a standard canola seed lot above the canola quality standard (i.e. <30 μmol g^{-1} of glucosinolate). Linear relationships were determined by taking into account the observed mean value of glucosinolate concentrations of contaminated seed samples and the mean values of uncontaminated seeds of Australian canola (i.e. 16 μmol g^{-1} of glucosinolate) reported by Seberry *et al*. (2016)*

Species	**Relationship†**	**Percent weed contamination (PWC) required for 30 micromole glucosinolate g^{-1} in admixture with oilseed rape**
Sinapis arvensis	y=1.80 x PWC + 16	7.78
Myagrum perfoliatum	y=1.67 x PWC + 16	8.38

Rapistrum rugosum	y=1.54 x PWC + 16	9.09
Carrichtera annua	y=1.46 x PWC + 16	9.59
Hirschfeldia incana	y=1.41 x PWC + 16	9.93
Conringia orientalis	y=1.24 x PWC + 16	11.29
Diplotaxis tenuifolia	y=1.19 x PWC + 16	11.76
Raphanus raphanistrum	y=1.19 x PWC + 16	11.76
Brassica fruticulosa	y=1.18 x PWC + 16	11.86
Brassica tournefortii	y=1.05 x PWC + 16	13.33
Diplotaxis muralis	y=1.02 x PWC + 16	13.73
Sisymbrium erysimoides	y=1.02 x PWC + 16	13.73
Diplotaxis tenuisiliqua	y=1.01 x PWC + 16	13.86
Brassica oxyrrhina	y=0.85 x PWC + 16	16.47
Sisymbrium officinale	y=0.79 x PWC + 16	17.72
Sisymbrium orientale	y=0.78 x PWC + 16	17.95
Sisymbrium irio	y=0.51 x PWC + 16	27.45
Capsella bursa-pastoris	y=0.47 x PWC + 16	29.79
Camelina sativa	y=0.28 x PWC + 16	50.00

*Uncontaminated oilseed rape seeds assumed to have a glucosinolate concentration of 16 μmol/g in oil-free oilseed rape meal based on the 2015 annual Australian Oilseeds Federation survey of crops (Seberry *et al.*, 2016).

†y, concentration of glucosinolates in seed admixture (30 μmol g^{-1}); PWC, percent weed contamination (based on model described by Davis *et al.*, 1999)

Table 4 Fatty acid profiles of weedy Brassicaceae species in comparison with average quality characteristics of Australian oilseed rape*. Mean and

standard error are presented for the different lines of each species (except for the three species that were represented by only one line)

Species	No. lines	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)	C20:1 (%)	C22:1 (%)
Brassica fruticulosa	5	3.9 ± 0.3	1.3 ± 0.1	8.1 ± 0.8	16.2 ± 1.1	12.8 ± 0.8	5.8 ± 1.0	45.0 ± 1.6
Brassica oxyrrhina	1	2.9	1.0	10.0	8.8	17.8	7.9	45.1
Brassica tournefortii	8	2.8 ± 0.3	1.3 ± 0.2	8.6 ± 0.6	11.5 ± 0.8	13.6 ± 1.1	7.3 ± 0.5	47.8 ± 1.9
Carrichtera annua	3	6.5 ± 0.4	0.6 ± 0.1	4.3 ± 0.1	14.8 ± 0.5	13.7 ± 0.6	1.9 ± 0.2	48.5 ± 0.4
Conringia orientalis	2	2.1 ± 0.0	0.4 ± 0.0	6.7 ± 0.1	26.2 ± 0.3	2.7 ± 0.2	26.3 ± 0.1	25.6 ± 0.6
Diplotaxis muralis	2	7.7 ± 0.2	2.1 ± 0.0	11.7 ± 0.6	22.8 ± 0.3	21.0 ± 0.3	6.7 ± 0.1	22.5 ± 0.6
Diplotaxis tenuifolia	7	4.7 ± 0.4	2.2 ± 0.1	20.3 ± 1.5	18.2 ± 1.3	23.9 ± 1.3	8.8 ± 0.4	17.1 ± 0.8
Diplotaxis tenuisiliqua	1	7.7	1.9	7.1	18.4	29.5	5.4	23.5
Hirschfeldia incana	9	5.8 ± 0.6	1.3 ± 0.3	10. 7 ± 0.8	11.0 ± 1.2	27.5 ± 2.6	5.7 ± 0.6	32.3 ± 4.8
Raphanus raphanistrum	10	5.5 ± 0.7	1.9 ± 0.3	20.8 ± 2.2	12.1 ± 1.4	12.3 ± 1.1	14.5 ± 1.0	31.5 ± 2.6
Rapistrum rugosum	7	4.7 ± 0.2	1.4 ± 0.2	9.3 ± 0.9	11.3 ± 1.2	21.6 ± 1.0	6.5 ± 0.6	36.4 ± 3.3
Sinapis arvensis	5	3.1 ± 0.4	1.0 ± 0.1	10.4 ± 0.8	12.4 ± 1.0	15.0 ± 0.5	13.3 ± 1.8	38.8 ± 1.1
Capsella bursa-pastoris	5	8.8 ± 0.3	4.2 ± 0.2	14.2 ± 1.3	20.6 ± 1.6	33.9 ± 1.7	12.2 ± 1.4	0.7 ± 0.1
Myagrum perfoliatum	2	6.0 ± 0.4	1.3 ± 0.0	12.3 ± 0.6	13.2 ± 0.5	30.4 ± 0.8	6.0 ± 0.1	25.6 ± 0.3
Camelina sativa	1	5.3	2.4	15.7	15.6	37.2	14.9	2.6
Sisymbrium erysimoides	2	10.4 ± 0.2	1.4 ± 0.0	7.6 ± 0.1	14.8 ± 0.2	30.9 ± 0.0	6.6 ± 0.0	20.4 ± 0.2
Sisymbrium irio	5	10.3 ± 0.7	2.3 ± 0.2	11.8 ± 2.0	15.5 ± 0.4	37.0 ± 0.9	7.4 ± 0.5	9.4 ± 1.9
Sisymbrium officinale	7	8.2 ± 0.4	1.2 ± 0.1	6.6 ± 0.7	12.5 ± 0.5	33.6 ± 1.6	5.7 ± 0.2	23.1 ± 2.3

Sisymbrium orientale	7	7.5 ± 0.5	1.0 ± 0.2	7.2 ± 0.6	11.0 ± 0.9	34.2 ± 0.6	5.8 ± 0.4	25.4 ± 1.5

*Average quality characteristics of Australian oilseed rape are 64.3% oleic acid (C18:1), 17.9% linoleic acid (C18:2), 8.6% linolenic acid (C18:3) and <0.1% erucic acid (C22:1) based on the annual Australian Oilseeds Federation survey of crops (Seberry *et al.*, 2016).

Table 5

Linear relationships between erucic acid concentration of a canola-weed admixture and percent weed contamination (PWC) for each weed species and the minimum PWC required to increase the erucic acid concentration of a standard canola seed lot above the canola quality standard (i.e. <2% of erucic acid). Linear relationships were determined by taking into account the observed mean value of glucosinolate concentrations of contaminated seed samples and the mean values of uncontaminated seeds of Australian canola (i.e. 0% erucic acid) reported by Seberry *et al*. (2016)*

Species	Relationship	Percent weed contamination (PWC) required for 2% erucic acid in admixture with oilseed rape*
Carrichtera annua	y=0.485 x PWC	4.12
Brassica tournefortii	y=0.478 x PWC	4.18
Brassica oxyrrhina	y=0.451 x PWC	4.43
Brassica fruticulosa	y=0.450 x PWC	4.44
Sinapis arvensis	y=0.388 x PWC	5.15
Rapistrum rugosum	y=0.364 x PWC	5.49
Hirschfeldia incana	y=0.323 x PWC	6.19
Raphanus raphanistrum	y=0.315 x PWC	6.35
Myagrum perfoliatum	y=0.256 x PWC	7.81
Conringia orientalis	y=0.256 x PWC	7.81
Sisymbrium orientale	y=0.254 x PWC	7.87
Diplotaxis tenuisiliqua	y=0.235 x PWC	8.51
Sisymbrium officinale	y=0.231 x PWC	8.66
Diplotaxis muralis	y=0.225 x PWC	8.89
Sisymbrium erysimoides	y=0.204 x PWC	9.80
Diplotaxis tenuifolia	y=0.171 x PWC	11.70
Sisymbrium irio	y=0.094 x PWC	21.28
Camelina sativa	y=0.026 x PWC	76.92
Capsella bursa-pastoris	y=0.007 x PWC	285.71

*Uncontaminated oilseed rape seeds assumed to have an erucic acid concentration of 0% based on the 2015 annual Australian Oilseeds Federation survey of crops (Seberry *et al.*, 2016).

†y, content of erucic acid in seed admixture (2%); PWC, percent weed contamination (based on model described by Davis *et al.*, 1999)

Table 6 Flowering and seed characteristics of weedy Brassicaceae species. Mean and standard error are presented for the different lines of each species (except for the three species that were represented by only one line)

Species	No. lines	Thousand seed weight (g)	Seeds/pod	Seed yield (g plant^{-1})	Height (cm)	Days to flowering	Pod shatter resistance (0–4)*
Sisymbrium irio	5	0.083 ± 0.008	73.2 ± 3.7	1.74 ± 0.25	115 ± 20	100 ± 5	0
Sisymbrium erysimoides	2	0.090 ± 0.001	55.0 ± 5.6	1.47 ± 0.10	130 ± 0	88 ± 3	0
Capsella bursa-pastoris	5	0.116 ± 0.016	24.5 ± 2.2	1.95 ± 0.60	85 ± 10	91 ± 15	0
Diplotaxis tenuisiliqua	1	0.154	19.6	1.13	115	88	0
Diplotaxis muralis	2	0.240 ± 0.039	39.0 ± 10.2	1.44 ± 0.32	73 ± 3	81 ± 1	0
Sisymbrium orientale	7	0.253 ± 0.021	177.0 ± 30.5	3.62 ± 0.86	112 ± 25	109 ± 7	3
Diplotaxis tenuifolia	7	0.332 ± 0.023	43.1 ± 8.9	0.55 ± 0.28	108 ± 10	159 ± 20	0
Sisymbrium officinale	7	0.352 ± 0.030	14.8 ± 1.7	3.54 ± 0.24	155 ± 10	129 ± 4	3
Hirschfeldia incana	9	0.356 ± 0.056	7.6 ± 1.4	2.18 ± 0.39	154 ± 15	156 ± 5	3
Brassica fruticulosa	5	0.601 ± 0.061	15.8 ± 2.9	2.41 ± 0.22	99 ± 18	92 ± 4	0
Camelina sativa	1	0.906	17.3	4.94	110	96	3.5

Rapistrum rugosum	7	0.977 ± 0.181	1.4 ± 0.5	1.57 ± 0.52	158 ± 20	123 ± 5	4
Brassica oxyrrhina	1	1.227	15.8	2.54	130	134	2.5
Brassica tournefortii	8	1.279 ± 0.116	19.7 ± 1.7	4.70 ± 0.89	88 ± 8	92 ± 10	3
Carrichtera annua	3	1.360 ± 0.165	5.0 ± 0.8	2.73 ± 0.48	70 ± 13	93 ± 7	4
Sinapis arvensis	5	2.255 ± 0.469	6.8 ± 2.2	3.07 ± 0.63	137 ± 15	102 ± 14	2
Conringia orientalis	2	2.548 ± 0.086	40.8 ± 0.1	3.54 ± 0.45	73 ± 3	109 ± 1	3.5
Myagrum perfoliatum	2	3.875 ± 0.361	1.0 ± 0.0	2.31 ± 0.39	85 ± 10	106 ± 7	4
Raphanus raphanistrum	10	4.730 ± 1.340	4.3 ± 1.1	2.87 ± 0.77	101 ± 30	111 ± 10	4

*Visual pod shattering resistance assessments were made at maturity on a 0 to 4 scale where 0 is very susceptible and 4 is indehiscent.

Appendix 1

Fertiliser details:

1.0 kg m^{-3} Osmocote 3-4 month time-release fertiliser (15:5.2:12.5 N:P:K);

2.0 kg m^{-3} Osmocote 8-9 month time-release fertiliser (18:4.8:8.3 N:P:K);

0.08 kg m^{-3} Iron chelate EDDHA (6% Fe);

1.0 kg m^{-3} 3-4 month time-release iso-butylidene-Di urea (31% N);

0.3 kg m^{-3} Trace element mixture (12% Fe, 2.5% Mn, 1.0% Zn, 0.5% Cu, 0.1% Bo, 0.005% Mo, 15% S).

Additional nutrients were supplied in a weekly watering with 2 g L^{-1} Aquasol liquid fertiliser (23:4:18 N:P:K).