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The effects of an altered glucosinolate profile, on the invertebrates within a *Brassica napus* crop.

by

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Summary

In this study double haploid (DH) *Brassica napus* plants containing novell allelles from wild *Brassica* species (1) were grown in a series of field trials, with the comercial *B. napus* cultivar Recital. The Glucosinolate (GSL) content and profile of the two plant types and volatiles produced from damaged tissue, were monitored throughout the growing. The effect of breeding for a novel GSL profile on yield and the effect of the altered GSL profile on the insect community of the crop were assessed.

The GSL profile and volatile signature of the DH plants was found to be distinct from that of the Recital plants. There was no indication that breeding for an altered GSL profile had affected yield. Regular sampling of the insect fauna of the crop using sweep netting and sticky traps showed that the early flowering DH plants were initially more attractive than Recital to several pest and beneficial species suggesting that it may function as a temporal trap crop. However, *Meligethes aeneus* (Pollen beetle) continued to be attracted to the DH plants throughout the season, suggesting an attraction to the GSL profile of the DH plants. Sampling also showed that DH plots contained higher numbers of non pest individuals and for much of the growing season higher levels of species richness than Recital plots.

The attractiveness of the DH to important pests such as *Ceutorhynchus assimilis* (the cabbage seed pod weevil) and *Meligethes aeneus* coupled with a yield comparable to many commercial cultivars suggests that the DH plants may make an excellent trap crop for winter OSR.

Background

Glucosinolates (GSLs) also known as mustard oils are the compounds which give *Brassica* species their characteristic smell and taste. In *Brassica* species GSLs and their breakdown products form the basis of a chemical defence system, and have been shown to have insecticidal (2, 3, 4), nematocidal (5, 6), bactercidal (7, 8, 9) antifungal (10, 7, 11), alleochemical (12, 13, 14) and even anticarcinogenic properties (15). *Brassica* species such as oilseed rape (OSR) are largely protected from attack by generalist herbivores by the presence of glucosinolates.

Glucosinolates are found throughout the plant (16) but are often concentrated in the newest growth (17). This offers maximum protection to the growing point of plant during winter and to the flowers and pods during the summer. If the plant is damaged, the GSLs which are stored in 'S' cells come into contact with an enzyme (myrosinase) stored in separate compartments called myrosin cells (18, 19). The enzyme startes to breakdown the GSL producing highly active secondary metabolites, such as isothiocyanates, epithionitriles and nitriles. It is these breakdown products which help to protect the plant.

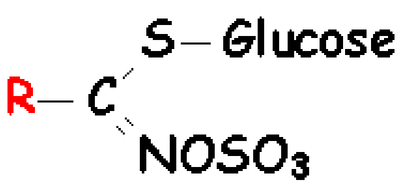

Figure 1. Generalised glucosinolate structure, with R representing the variable side chain.

The unappetising and toxic breakdown products of glucosinolates will usually deter generalist herbivores from feeding. However, if they are consumed the toxic and anti-nutritive effects can be clearly seen. This is perhaps most obvious in livestock fed on high GSL rapeseed meal which may develop goitres (20), although less obvious the effect of GSL breakdown products on invertebrate herbivores can be just as negative (21). Despite their toxicity to many species certain insect herbivores have developed mechanisms to detoxify GSL breakdown products and overcome the plants primary defence (22). Many of these insects such as the cabbage aphid and the diamond back moth now feed almost exclusively on *Brassica* species, and actively use the GSLs as feeding and egg laying cues (23, 24, 25). The plants, however, are not entirely without protection from these specialists as GSLs also provide the plant with a second level of defence by attracting beneficial insects such as parasitoids and predators which attack the pests to the plants (26, 27, 28).

Modern oilseed rape is the product of numerous breeding programmes which have sought not only to improve general agronomic characters such as yield, but also to reduce the levels of anti-nutritive compounds in the oil and meal. As a result the GSL profiles of the most widely grown varieties of winter OSR are relatively uniform (29). These modern OSR cultivars lack both the variety and concentrations of GSLs found in wild *Brassica* species and this may have had a detrimental effect on their protection from pests and pathogens. Resistance to pests and disease is often more pronounced in wild species. In potato species, For instance, high levels of resistance to the peach potato aphid have been identified in wild species whereas no resistance was displayed in over 360 cultivated accessions (30). Similarly, insect resistance in cultivated tomato is rare, but more common in wild accessions (31). In this study OSR plants containing genetic material from wild *Brassica* species (1), were used to assess the impact of a different glucosinolate profile on important pests, beneficial insects and yield.

The double haploid OSR plants used in this study are the result of a breeding programme which began with crossing a wild *Brassica rapa* from Sicily with a wild form of *Brassica oleracea* from Tunisia (1). The wild *B. rapa* and *B. oleracea* had very different GSL profiles to commercial *B. napus* plants, which primarily contain pentenyl GSLs Which has a 5 carbon side chain. The wild *Brassica* species contained high levels of but-3-enyl which has a 4-carbon side chain, and prop-2-enyl which has 3-carbon side chain (1). Like their wild ancestors the double haploid *B. napus* plants produced as a result of this breeding programme also contained high levels of 3- and 4-carbon.

Initial investigations during previous work had shown that the novel GSL profile may affect insects within the crop. DH plants with increased levels of 4 carbon GSLs were shown to be attractive to the aphid parasitoid *Diaeretiella rapae* (34), whereas the cabbage stem flea beetle *Psylliodes chrysocephala*, showed a preference for plants with high levels of the 3 carbon GSL (32). This study follows on from these findings and aimed to assess the effect of a novel GSL profile on the insect community of the crop as a whole.

Field trials

Three field trials were undertaken near Sutton Bonington, Nottinghamshire, U.K., between September 2002 and August 2005. The trials were managed conventionally using best farm practice with the exception that no insecticide treatments other than a Cypermithrin spray in December were used in the first two trials. During the first trial a single DH line was selected to be used with the commercial cultivar Recital (Syngenta seeds). This DH line was used in the second trial in a variety of mixes with Recital in an attempt to produce a GSL dose effect and assess the impact this had on the invertebrate fauna. The third trial used the same seed mixes to look at the effect of yield.

Glucosinolate extraction and identification

GSL extraction was achieved by the conversion of GSLs to desulphoglucosinolates (DSGSL) which where then eluted from an ion exchange column (33). The DSGSLs were separated on a reverse phase HPLC column and identified with reference to standards identified by Heaney (33).

Insect sampling

Sweep samples and sticky traps were used throughout the year to monitor insect activity. A yellow double-sided sticky trap 20cm in size (Monroe) was placed in the centre of each plot just above the canopy and changed every seven days. Traps were covered in cling-film when collected and stored at 2ºC. Sweep sampling began on the 31/03/04, samples, consisting of 15 swipes were taken from each plot once or twice weekly, as weather permitted. Sweep samples were then stored at -20ºC until they could be processed, insects were then stored in 70% ethanol.

Findings

Glucosinolate profiles

A comparison of three DH lines with the commercial cultivar Recital in the 2002-3 field trial indicated that DH line 10212 produced the highest butenyl concentrations and this line was selected for use in the remainder of the study. The GSL content of Recital plants is composed of primarily aliphatic GSLs such as pentenyl and OH butenyl (Figure 2). The DH plants produce mainly butenyl GSLs and significantly less OH Butenyl, pentenyl and OH pentenyl than Recital plants (Figure 2). Although present at much lower concentrations, there are also differences in the levels of indole and aromatic (phenylethyl) GSLs.

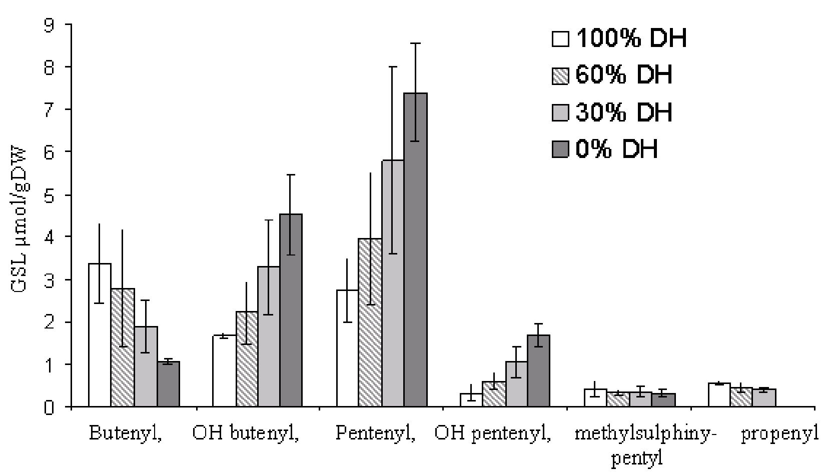

Figure 2. Aliphatic GSL levels in leaf tissue sampled in January 2004.

The differences in the GSL profiles of the two plant types made it possible to produce four treatments using seed mixes of DH and Recital seed which differed in both total GSL content and GSL profile (Figure 3).

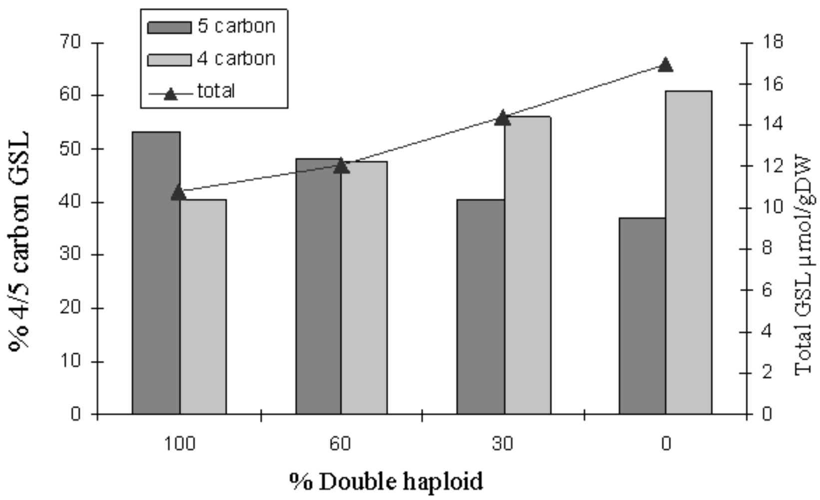

Figure 3. Percentage four-and five-carbon GSLs and total GSL present in leaf tissue from the four seed mixtures.

Pest species

Pollen beetles *Meligethease* spp.

Pollen beetle larvae were caught from 20/04/04 and peaked in numbers during May (Figure 4). Catches of pollen beetle larvae showed a highly significant relationship the DH content of the plot ($P = 0.011$). Plots containing 100% and 60% DH plants contained higher numbers of pollen beetle larvae throughout the sampling period than plots containing 100% Recital and 0% DH seed (Figure 4).

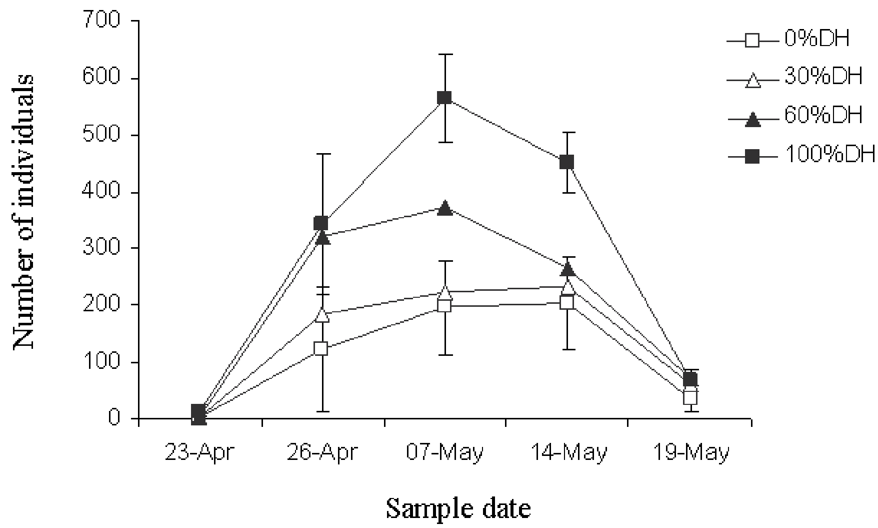

Figure 4. Mean numbers of pollen beetle larvae caught in sweep samples taken from plots containing 0, 30, 60 and 100% double haploid seed. Error bars on 100% DH and 0% DH plot data = ±1 stdev

Adult pollen beetle numbers reached a peak during late April, adults were then almost absent from the crop until mid to late June when they could be seen swarming on any remaining flowers and the yellow sticky traps. Analysis showed that sticky trap catches of adult pollen beetles exhibited highly significant variation with the double haploid content of the plot (P = 0.038). Sweep net catches of adult pollen beetles were also significantly affected by the DH content of the plot (P = 0.048).

Weevils *Ceutorhynchus* spp.

Weevils of the genus *Ceutorhynchus* primarily the Cabbage Seed Weevil, *Ceutorhynchus assimilis,* accounted for the majority of weevils within the crop, however, the cabbage stem weevil, *Ceutorhynchus pallidactylus,* was also found. Analysis of sweep catches showed that catches were significantly affected by the DH content of the plot (P=0.086).

Ceutorhynchus spp showed a similar preference to pollen beetles with more being caught in DH plots than Recital plots. As with the pollen beetles the weevils also seemed to be sensitive to the GSL dose of the plots; with higher catch numbers in the 60% DH plots compared to the 30% DH plots (Figure 5).

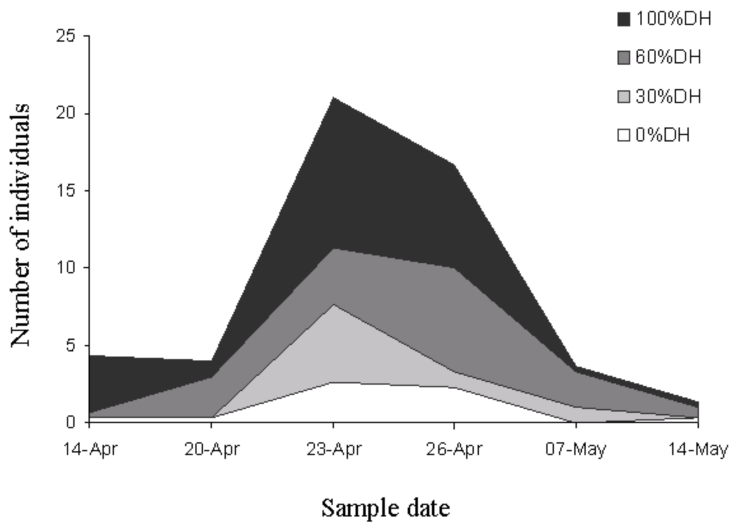

Figure 5. Mean numbers of *Ceutorhynchus* weevils caught in sweep samples collected from the four treatments between the 14th April and the 14th of May 2004, data is stacked.

Beneficial species

In addition to pest species the numbers of beneficial invertebrates predators and parasitoids within the crop were also monitored. The most abundant of theses were the parasitic wasps, although, *arachnids*, and other beneficials such as lacewings, rove beetles, and ladybirds were also caught. Figure 6 below shows the number of parasitic wasps caught in plots with varying DH content.

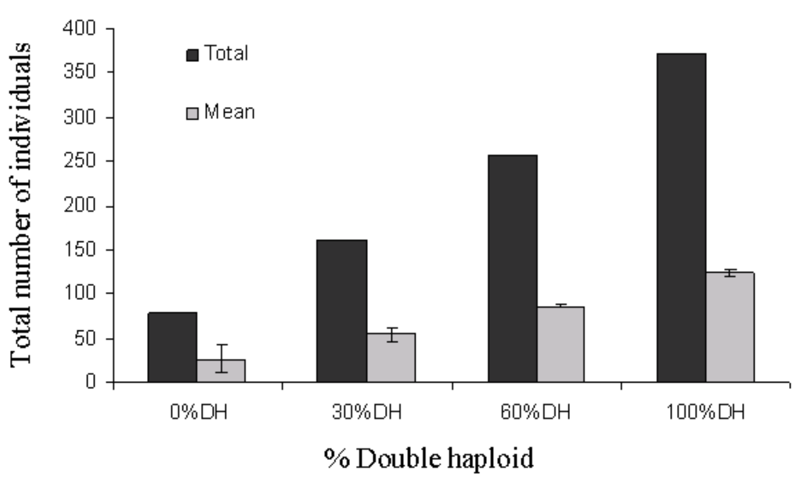

Figure 6. Total and mean number of parasitic wasps caught in sweeps collected from each treatment type in 2004, error bars = ±1 stdev.

A greater total number of parasitic wasps were caught in DH plots compared to Recital; however, the attraction of the DH plots to the wasps seemed to vary with time. Figure 7 shows that between the 14-21st of May the DH plots were more attractive to parasitic wasps than Recital plots. However during the 24th-31st of May this trend is reversed as Recital plots become the most attractive to the wasps.

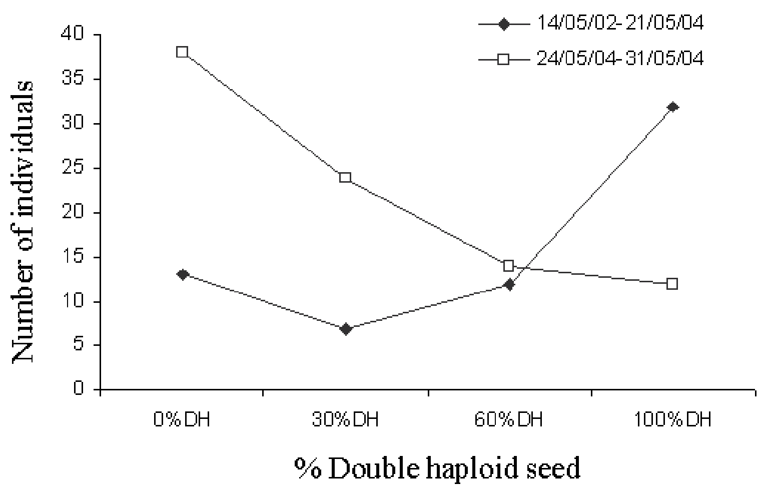

Figure 7. Total number of ichneumonid wasps caught on sticky traps set on the 14/05/02 and on the 24/05/04.

Community effects

Specialist pest species such as pollen beetles and weevils together with beneficial species of parasitic wasps comprised a very large proportion of the invertebrate community of the crop. However, the crop also contained populations of generalist herbivores and predators (Table 1).

	Type	**Total caught in sweeps**
Predators	Staphylinidae	10
Predators	Coccinelidae	2
Seed/plant feeders	Bruchidae	6
Mould feeders	Lathridiidae	4
Predatory	Cantharidae	4

Generally predatory	Coleoptera larvae	1
Herb/Pred	Heteroptera	5
Herb/Pred	Homoptera	1
Predatory	Neuroptera	2
Herbivores	Symphyta	1
Herb/Pred	Nematocera	281
Herb/Pred	Brachycera and Cyclorrhapha	260
Herb/Pred	Diptera larvae	8
Herbivores	Thrips	19
Herbivores	Lepidoptera adults	0
Herbivores	Lepidoptera larvae	1
Predatory	Spiders	27

Table 1. Invertebrate community of trial crop, (excluding Meligethes, Ceutorhynchus, Phyllotreta species, aphids and parasitic wasps).

The level of species richness in the crop was low during early spring and peaked during flowering, gradually declining as the crop finished flowering and matured.

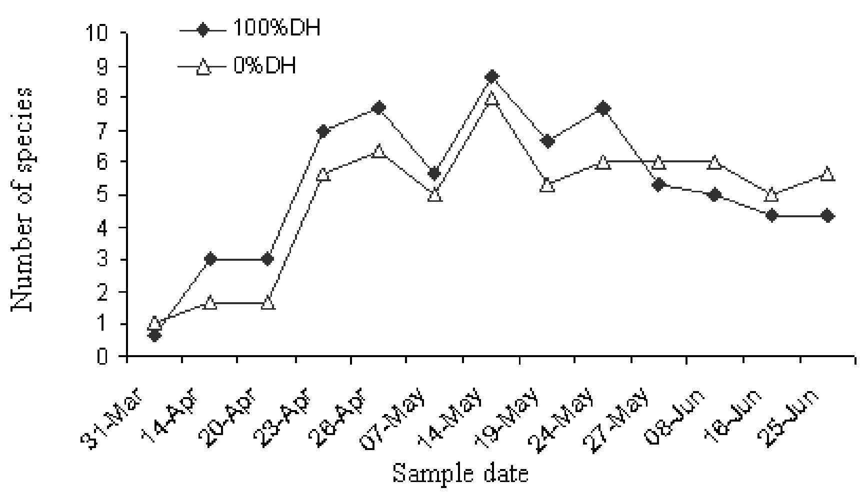

Figure 8. Mean levels of species richness found in DH and Recital plots, collated using sweep sample data from 2004

During much of the growing season the DH plants have a higher level of species richness (Figure 8).

This difference is well illustrated by comparing the number of non-pest coleopteran species caught in sweep samples collected from DH and Recital plots. DH plots contained a more complex community with five non-pest species found compared to just two in Recital plots. Plots containing DH and Recital seed mixes showed an intermediate level of diversity (Figure 9).

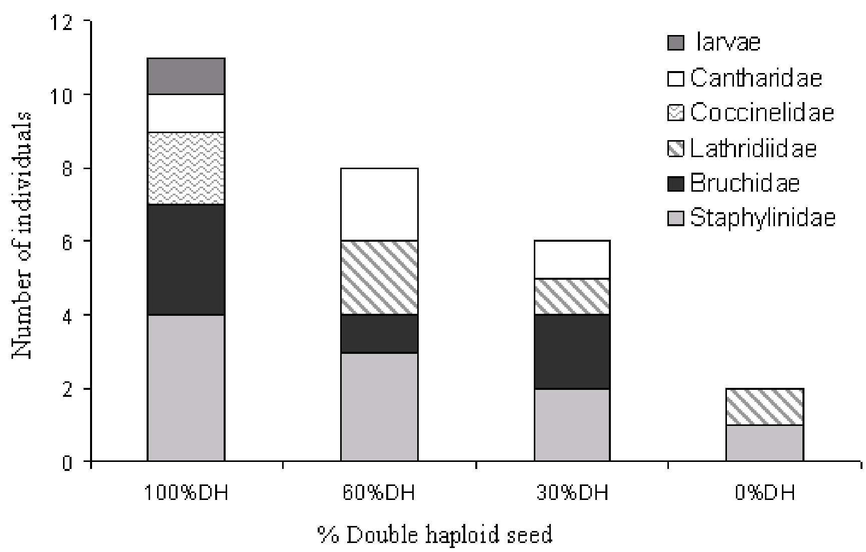

Figure 9. Non-pest Coleoptera, total number of individuals caught in sweep samples, excluding Meligethes, Ceutorhynchus and Phyllotreta species

Effect of glucosinolate content on yield

Combine harvester yields collected from the 2004-05 trial showed that overall trial plots produced an average yield of 4.32 tons per hectare. Recital plots produced an average yield of 4.22t/ha and DH plots 4.34t/ha (Figure 10).

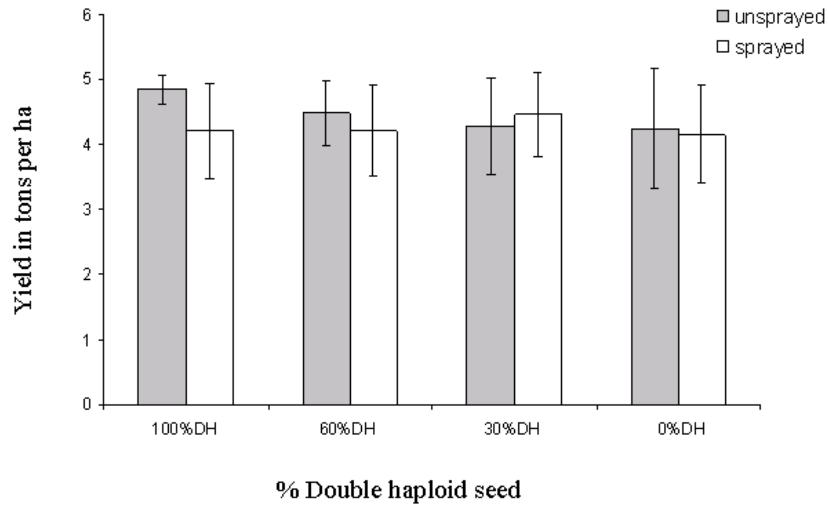

Figure 10. Mean yield in tons per hectare from each treatment type + st dev, sprayed (treated with insecticides) and unsprayed (no insecticide treatment)

An ANOVA showed there was no significant difference in yield between the four seed treatments (P = 0.969). The average yield from sprayed plots was 4.25t/ha and from un-sprayed plots 4.38t/ha indicating a slight increase in the yield of unsprayed plots with increasing DH content (Figure 10). However, this difference was very small and when analysed with a two way ANOVA not significant (P=0.955).

Conclusions

Glucosinolate profiles

Of the three double haploid lines used in the 2002-03 trial, line 10212 produced the highest levels of butenyl GSL and was selected for use in the remainder of the study. As expected from its high seed GSL content (HGCA recommended list 2002/2003) the commercial cultivar Recital provided a good contrast to the DH plants, producing significantly higher total GSL levels but lower levels of 3- and 4- carbon GSLs such as butenyl. The GSL profile of Recital plants is dominated by the five-carbon aliphatic GSL pentenyl whereas the DH plants contain more four-carbon butenyl than pentenyl. The Recital plants also contain higher levels of OH-butenyl and OH-pentenyl: the hydroxylated forms of these GSLs. In general, the DH plants produce higher levels of the indole GSLs such as OH indolylmethyl, methoxyindolylmethyl, 1-methoxyindolylmethyl than Recital. Recital plants produced higher levels of the aromatic GSLs phenylethyl produced from the amino acid phenylalanine.

Specialist species- Pollen beetles

Pollen beetles, weevils, and flea beetles were the most common pests caught in the 2004 trial, aphids were also common, predominantly *Brevicoryne brassicae*. Sticky trap and sweep samples both demonstrated that some pest species showed a preference for DH plots over Recital. This response was particularly strong in pollen beetles suggesting that they are attracted to the increased levels of butenyl ITC produced by the DH plants. Pollen beetles also seemed sensitive to the GSL dose of the plot, with catches increasing with DH seed content. Results indicate that adult pollen beetles found the DH plants to be an attractive host plant, as significantly higher numbers of larvae were caught in DH plots than in Recital plots with mixed plots producing intermediate levels.

Generalist species and species richness

The *B. napus* crop contained a range of generalist species primarily predatory and nectar feeders, with very low levels of generalist herbivores. There is some evidence to suggest that the DH profile of the plants may have affected invertebrates such as spiders. However, as many of these species are predators their distribution is more likely to be correlated with that of their prey. The level of species richness in the crop was low during early spring and peaked during flowering, gradually declining as the crop finished flowering and matured. For much of the growing season the DH plots had a higher level of species richness. The difference in species richness suggests that DH crops or plots may offer a more diverse invertebrate fauna and greater numbers of beneficial insects. This may be due to lower levels of total GSL in the plants making the herbivores feeding on them more palatable, a function of the increased number of prey items such as pollen beetle larvae or a combination of the two.

Hymenopteran parasitoids

The DH content of field trial plots had a significant effect on both sweep and sticky trap catches of parasitic wasps, increasing the total number of wasps caught in DH plots. This DH plants were much more attractive to certain types of wasps and a dose response was seen with wasp numbers declining with DH content. However, the difference in growth rate of the DH and Recital plants coupled with the specific larval and plant stages favoured by several species of wasp, for example attacking pollen beetle larvae, complicate a direct comparison of the two plant types. Nonetheless, it is possible that the number of wasps in Recital plots between the 24/05/04 and the 31/05/04 was increased by the presence of the DH plants. Wasps are likely to have been attracted into the crop by the DH plants, which flowered earlier and therefore contained the appropriate-aged larvae to parasitise before the Recital plants. This may have allowed the number of wasps to build up and therefore a larger number of parasitic wasps were already present within the crop when the Recital plants and their associated pollen beetle larvae reached the correct developmental stage.

Trap crops

The attractiveness of the DH plants to specialist pests such as the pollen beetle suggests that certain DH lines have considerable potential as a trap crop. Research has shown that turnip rape (*Brassica rapa* L.) can be an effective trap crop for spring sown OSR crops. Turnip rape has been shown to be attractive to a range of *Brassica* pests such as the pollen beetle and cabbage seed pod weevil (34, 35, 36). Cook found that pollen beetles remained on turnip rape until the main oilseed rape crop was well past the yellow bud stage, which is most susceptible to damage by pollen beetle (37). Cook also found that seed weevil numbers remained low on oilseed rape plots compared to turnip rape (37). Turnip rape has also been shown to be effective in attracting stem-mining pests such as the cabbage stem flea beetle (38). It has been suggested the success of turnip rape as a trap crop is due to its earlier flowering with respect to oilseed rape and its more attractive odour (37). Turnip rape is an excellent candidate as a trap crop in spring OSR however a viable trap crop for winter OSR has yet to be found (39).

The DH plants have several characteristics, which indicate it may make an excellent trap crop for winter OSR. They are preferentially attractive to pests such as the pollen beetle both due to their novel GSL profile and their early flowering time and possibly their lighter leaf colour. The DH plots contained significantly higher numbers of pollen beetle adults and larvae throughout the trial, but particularly in early April. It is in the early stages when plants are in bud that pollen beetles and their larvae can cause the most damage to a crop (40, 41). As the DH plants flower earlier and are more attractive to pollen beetles it is likely that a trap crop of DH plants sown around the perimeter of an OSR crop could retain the beetles whilst the main crop was at its most vulnerable stage.

As headlands often produce lower yield than central areas of a field, sowing these areas with a trap crop may not impact greatly on yield. This may be particularly true for a DH trap crop as the DH plants produce a comparable yield to commercial cultivars of winter OSR. Although there is no yields data available for winter turnip rape, average yields of 2.20 t/ha for spring turnip rape are approximately 10-15% lower than spring OSR yields (42). This indicates that a winter turnip rape may also produce a lower yield than commercial winter *B. napus* cultivars and the DH plants used in this study.

This study has shown that an altered GSL profile in *B. napus*, which includes natural enemy enhancing traits such as elevated levels of butenyl GSL, can be introduced without negatively impacting on yield.

References

1. **Bradburn R P 2000,** The formation and function of Glucosinolates in *Brassica napus* PhD Thesis submitted to the University of East Anglia**.**

2. **Rodman, J. E., Chew, F. S., 1980,** Phytochemical correlates of herbivory in a community of native and naturalized cruciferae. Biochemical Systematics and Ecology 8: 43-50

3. **Mithen, R., Heany, R. K., Fenwick, G. R., 1986,** Invitro activity of glucosinolates and their products against *Leptosphaeria maculans.* Transactions of the British Mycological Society 87: 433-440

4. **Hammond K E, Lewis B G, 1987,** The establishment of systemic infections in leaves of oilseed rape by *Leptosphaeria maculans.* Plant pathology 36: 135-147

5. **Lazzeri, L., Tacconi, R., Palmieri, S., 1993,** Invitro activity of some glucosinolates and their reaction products towards a population of the nematode Heterodera schachtii. Journal of Agricultural and food chemistry 41: 825-829

6. **Mayton, H. S., Oliver, C. Vaughn, S. F., Loria, R., 1996,** Correlation of fungal activity of Brassica species with allyl isothiocyanate production in macerated leaf tissue. Phytopathology 86: 267-271

7. **Delaquis, P J, Mazza G, 1995,** Antimicrobial properties of isothiocyanates in food preservation. Food Technology 49: 73-79

8. **Hashem F A, Saleh M M , 1999,** Anti microbial components of some cruciferae plants (Diplotaxis harra Forsk and Erucaria microcarpa Boiss. Phytotherapy Research 13: 329-332

9. **Lin, C. M., Kim, J., Du W. X., Wei, C. I., 2000,** Bacteriocidal activity of isothiocyanates against pathogens of fresh produce Journal of food protection 63: 25-30

10. **Drobinca L, Zemanova M, Nemec P, Antos K, Kristian P, Stullerova A, Knoppova V, Nemen P, 1967,** Antifungal activity of isothiocyanates and related compounds naturally occurring isothiocyanates and their analogues. Applied Microbiology 15: 701-709

11**. Mari, M., Iori, R., Leoni, O., Marchi, A., 1993** In vitri activity of glucosinolate-derived isothiocyanates against post-harvest fruit pathogens. Annals of Applied Biology 123: 155-16

12. Brown P D, Morra M J, 1995 Glucosinolate-containing plant tissues as bioherbicides. Journal of Agricultural and food Chemistry 43: 3070-3074.

13. Charron C G, Sams C E, 1999, Inhibition of *Pythium ultimum* and *Rhizoctonia solani* by shredded leaves of Brassica species. Journal of the American Society of Horticultural science 124: 462-467

14. Smith, V. L., 2000, Reduction in snap bean emergence by seed treatment with dried Canola residue. Hortscience 35: 92-94

15. **Mithen, R., 2001**. Glucosinolates - biochemistry, genetics and biological activity. Plant Growth Regulation. 34: 91-103.

16. **Fahey JW Zalcmann AT Talalay P 2000** The chemical diversity and distribution of glucosinolates and isothiocyanates among plants Phytochemistry 56: 5-51

17. **Lambdon, P. W., Hassall, M., Boar, R. R., Mithen, R., 2003,** Asynchrony in the nitrogen and glucosinolate leaf-age profiles of Brassica: is this a defensive strategy against generalist herbivores? Agriculture Ecosystems and Environment 97: 205-214

18. **Kelly, R. J., Bones, A., Rossiter, J. T., 1998.** Subcellular immunolocalisation of the glucosinolate sinigrin in seedlings of *Brassica juncea.* Planta 206: 370-377

19. **Koroleva, O. A., Davies, A., Deeken, R., Thorpe, M. R., Tomos, A. D., and Hedrich R., 2000,** Identification of a new glucosinolate rich cell type in Arabidopsis flower stalks. Plant Physiology. 124: 599-608

20. **Chew F S 1988,** Biological effects of glucosinolates in biologically active natural products: potential use in agriculture. pp 155-181 Ed, H G cutler, Washington DC American chemical society.

21. **Blau P A, Feeny P, Contardo L Robson D S 1978,** Ally glucosinolate and herbivorous caterpillars a contrast in toxicity and tolerance. Science 200: 1296- 1298

22. **Ratzka, A., Vogel, H., Kliebenstein, D.J., Mitchell-Olds, T., Kroymann, J. 2002**, Disarming the mustard oil bomb. Proceedings of the National Academy of Sciences USA 99: 11223-11228

23. **Bartlet E, Parsons D Williams I H Clarke S J 1994,** The influence of glucosinolates and sugars on feeding by the cabbage flea beetle *Psylliodes Chrysocephala* Entomologia Experimentalis et Applicata 73: 77-83

24. **Bodnaryk R P, 1997,** Will low glucosinolate cultivars of the mustards *Brassica juncea* and *Sinapis alba* be vulnerable to insect pests? Canadian journal of Plant Science 77: 283-287

25. **Vinson, S. B., Elzen, G. W. & Williams, H. J. 1987.** The influence of volatile plant allelochemicals on the third trophic level (parasitoids) and their herbivorous hosts. In *Insect–Plants*, eds. V. Labeyerie, G. Fabres & D. Lachaise, pp. 109–114. Dordrecht:Junk.

27. **Dicke, M., van Beek, T.A., Posthumus, M.A., Ben Dom, N., van Bokhoven, H. and De Groot, A.E. 1990.** Isolation and identification of

volatile kairomone that affects acarine predator-prey interaction, involvement of host plant in its production. Journal of Chemical Ecology 16: 381-396.

28. **Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. 1990.** Exploitation of herbivore induced plant odors by host-seeking parasitic wasps. Science 250: 1251-1253.

29. **Fieldsend J, Milford G J F,** 1994 Changes in glucosinolates during crop development in single and double low genotypes of winter oilseed rape: I production and distribution in vegetative tissues and developing pods during development and potential role in the recycling of sulphur within the crop. Annuals Applied Biology 124: 531-542

30. **Flanders K L, Radcliffe E B, Hawkes J G, Lauer F I, 1992,** Insect resistance in potatoes: sources, evolutionary relationships, morphological and chemical defenses and ecogeographical associations. Euphytica 61: 83–111.

31. **Eigenbrode, S. D., T. Castagnola, M.-B. Roux, and L. Steljes. 1996.** Mobility of three generalist predators is greater on cabbage with glossy leaf wax than on cabbage with a wax bloom. Entomologia Experimentalis et Applicata 81: 335-343.

32. **Bradburn R P Mithen R 2000,** Glucosinolates genetics and the attraction of the aphid parasitoid *Diaeretiella rapae* to *Brassica*. Proceedings of the Royal Society London B 267, 89-95

33. **Heaney R K, Spinks A B, Hanley A B, Fenwick G R, 1986,** Analysis of glucosinolates in rapeseed. Technical bulletin, AFRC Food Research Institute, Norwich UK.

34. **Buechi R. 1990** Investigations on the use of turnip rape as trap plant to control oilseed rape pests. Bulletin IOBC/WPRS. 13: 32-39

35. **Hokkanen H. M. T., Granlund H., Husberg G.-B. And Markkula M. 1986** Trap crops used successfully to control *Meligethes aeneus* (Col., Nitidulidae), the rape blossom beetle. Ann Entomol. Fenn. 52: 115-120

36. **Cook S.M. Smart L.E., Potting R.J.P., Bartlet E., Martin J.L., Murray D.V., Watt N.O., Williams I H, 2002a** Turnip rape (*Brassica rapa*) as a trap crop to protect oilseed rape (*Brassica napus*) from infestation by insect pests: potential and mechanism of action. Proceedings of the BCPC Conference: Pest and Diseases, 2, 2002, British Crop Protection Council, Farnham, UK. Pp. 569-574.

37. **Cook S.M., Bartlet E, Murray DA, Williams I H 2002** The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. Entomologia Experimentalis et Applicata 104: 43-50

38. **Barari H, Cook S M, Clark S J. and Williams I H. 2005** Effect of a turnip rape *(Brassica rapa)* trap crop on stem-mining pests and their parasitoids in winter oilseed rape *(Brassica napus).* BioControl 50: 69 - 86

39. **Powell, W. 1986** Enhancing parasitoid activity in crops. In: D.J. Greathead & J. Waage (Eds.) Insect Parasitoids (pp.319-340). Academic Press, London.

40. **Nilsson, C. 1987**. Yield losses in summer rape caused by pollen beetles *(Meligethes* spp.). Swedish Journal of Agricultural Research 17: 105-11

41. **Alford 2000** Biological control of insects on oilseed rape in Europe Pesticide Outlook 11: 200-202

42. **Crowley, J.G. 1998.** The potential of new crop introductions. The Irish Agriculture and Food Development Authority. http://www.teagasc.ie/research/reports/crops/4164/eopr-4164.htm>