

Pod shatter resistance in the resynthesized *Brassica napus* line DK142

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SUMMARY

Resistance to pod shatter was studied within and between populations of the resynthesized *Brassica napus* line DK142, grown under glass or in the field, at sites in the UK and Belgium. All populations showed similar ranges of resistance that were greater than that of a commercial cultivar (Apex). The increase in range was at least three-fold greater than the range found in Apex. Only sowing time affected the descriptive statistics of shatter resistance of each line, with spring-sown populations more shatter susceptible than winter-sown populations. The partitioning of dry matter to individual tissues of the pod was different for the two lines, with dry matter biased to the seed in Apex and to the receptacle in the more resistant DK142. In DK142 and Apex, as well as F₁ and F₂ populations derived from crosses between DK142 × Apex, correlations for pod shatter resistance and mature pod characters were high, particularly the weight and length of the valves and septum. Shatter resistance increased in plants when pod numbers were reduced by the removal of whole racemes.

INTRODUCTION

Oilseed rape (*Brassica napus*) is a relatively undeveloped crop compared with cereals and pulses. It has many weed-like characteristics, including pods that open (shatter) easily when ripe. Although of benefit to a wild species, this trait is highly detrimental in a crop, with considerable amounts of seed lost prior to harvest during adverse weather conditions and on contact with the combine during harvesting. Losses are typically quoted as 10% (Kadkol *et al.* 1984), but estimated between 20% (Price *et al.* 1996) to as high as 50% if harvesting is delayed (MacLeod 1981; Child & Evans 1989).

The anatomy of the pod relating to the process of opening has been described by Picart & Morgan (1984) and Meakin & Roberts (1990). Oilseed rape pods consist of two valves, each containing seeds, on both sides of a false septum. The valves of fully

mature pods separate along dehiscence zones (DZs) which are situated within sutures that extend from the junction of the pod with the pedicel to the junction with the shrivelled style (beak). Current husbandry aims to reduce shatter by swathing or by the use of desiccant sprays just prior to full pod maturity. Both techniques desiccate and kill cells in the DZ preventing completion of the active process of cell separation. However, precise treatment timing in relation to stage of crop development is difficult to assess and asynchronous pod development, which results in differences in the time of seed maturation, can affect seed quality. Increased shatter resistance should reduce husbandry inputs, improve seed recovery and may also enhance seed quality. Moreover, less outlay will be needed to control volunteer plants in subsequent crops and seed losses outside the field boundary should be reduced providing an added ecological benefit.

To some extent, the amount of shatter is influenced by the canopy architecture. Pods that are held erect in the canopy are thought to be more protected than

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Pods that are horizontally orientated. Matting together of flexible racemes during the later stages of seed filling is also thought to reduce shatter (Thompson & Hughes 1986). However, although the canopy structure might affect efficiency of seed recovery, individual components of the canopy architecture could not be associated significantly with the inherent susceptibility of pods to shatter when they were tested away from canopy influences. The results of Morgan *et al.* (1998) showed that determinants of raceme morphology, such as pod angles, raceme length, thickness and width correlated poorly with pod shatter. However, there appeared to be a tendency for tall plants with thick stems to be more shatter resistant.

Although estimates of overall shatter status have been made in field crops of commercial cultivars, it is possible that variation in shatter values exists between plants within the populations. However, shatter susceptibility appears to be fairly uniform between individual plants within commercial cultivars and the range of variation, which has not yet been reported, may be quite small. It might be of advantage to breeders to identify individuals with higher than average shatter resistance in order to determine the architectural structure of the pod that is associated with difficulty of valve separation which results in reduced seed loss.

In contrast with the current situation in commercial cultivars, the variation in shatter resistance identified within the amphidiploid species *B. juncea* and in *B. carinata*, as well as in *B. oleracea* and *B. rapa*, the putative diploid ancestors of amphidiploid *B. napus*, appears to be much greater, and individuals with high shatter values are known to exist (Bowman 1984; Downey & Robbelen 1989). Synthetic lines of *B. napus* derived from interspecific crosses between *B. oleracea alboglabra* and *B. rapa chinensis* have been reported to contain a wide variation in shatter resistance (Morgan *et al.* 1998). Several individuals were found to have high shatter resistance values when measured by a standard test procedure. Genes for shatter resistance acted recessively and correlations with most pod and plant morphological characters were low, suggesting that it would be feasible to introgress the shatter-resistant trait into commercial breeding lines (Morgan *et al.* 2000).

The work reported in the present paper aimed to identify within the shatter-resistant, synthetic line DK142 and a more shatter-susceptible commercial cultivar Apex: (i) plant to plant variation in shatter values; (ii) the stability of the resistance trait of DK142 in different environments; and (iii) the possible relationship of shatter status with pod characters associated with valve separation. Shatter resistance was also determined in populations derived from crosses between DK142 and the commercial cultivar.

MATERIALS AND METHODS

Plant material

The *B. napus* line DK142 is derived from a selfed, doubled haploid, microspore-cultured plant that was itself derived from a cross between the doubled haploid winter breeding line N-0-109 (CPB Twyford Ltd, Hertfordshire, UK) and a synthetic interspecific hybrid of wild *B. oleracea alboglabra* and *B. rapa chinensis* at the John Innes Centre (Morgan *et al.* 2000).

Seed of DK142 for sowing in 1999 was produced from self-pollinated second-generation DK142 plants derived from the original microspore plant. Seed was bulked up in 2000 from cuttings taken from the second-generation DK142 plants. The shatter-susceptible commercial cultivar Apex was used as a standard comparison in all experiments. DK142 was cross-pollinated with Apex in May 1999 in order to provide seed for sowing early in August for the production of F₁ plants in a heated glasshouse. The F₁ plants were selfed to produce seed in time for raising F₂ plants in the field during normal outdoor seasonal conditions.

Assessment of pod shatter resistance

Pod shatter was assessed by a 'random impact test' (RIT). This is a reproducible and controlled means of assessing pod shatter and aims to mimic conditions in the crop canopy during agitation by weather or machinery (Bruce *et al.* 2002). As the lignocellulosic property of the valves makes them hygroscopic and their water content can affect shatter, pods were equilibrated for at least 3 days at 25 °C and 50% RH in a controlled environment cabinet before testing. For the test, 20 undamaged pods per plant were placed together with six steel balls of 12.5-mm diameter in a 20-cm diameter cylindrical container. The container was then mechanically shaken at a frequency of 4.98 Hz and a stroke length of 51 mm for two 10 s periods, followed where required by one period each of 20, 40 and 80 s. The times were chosen to give equal intervals after log transformation. At the end of each period, pods were examined and classed as shattered if one or both of the valves had detached. Using GenStat (Lawes Agricultural Trust, IACR-Rothamsted), the transformed data were fitted to a linear model and the time (s) taken for 50% of the pods to shatter (RIT₅₀) estimated, using a common slope and separate intercept model in sets of data grouped by commonality.

Morphological characterization and shatter resistance amongst populations of DK142 and Apex

DK142 and Apex seed was sown at the IACR Long Ashton Research Station in John Innes No. 1 potting compost in December 1999. Seedlings were transferred to John Innes No. 2 compost in 15-cm diameter

pots before vernalization at the four-leaf stage at 6 °C for 8 weeks. The plants were then set out in March in an unheated glasshouse in seven blocks, each of which contained 16 plants of DK142 and Apex.

Stem height, measured as the distance from soil level to the base of the terminal raceme, was recorded at weekly intervals after the start of stem elongation in early March. Flowering began in April. Pollination was enhanced by bees from hives situated within the glasshouse, and by hand pollinations. Approximately 5 weeks after the start of flowering only a few flowers on the lowest primary raceme and on secondary racemes remained open and all pods on the terminal and first primary racemes had extended to their final length. At this time, two plants per line were chosen from each of the seven blocks of plants for uniformity on the basis of set and vigour. On each plant ($n = 14$), all but the terminal and first primary racemes were removed and vegetative regrowth and secondary flowers were removed at weekly intervals.

All pods were fully mature by the last week of July. Pods were collected from the terminal and first primary raceme of all plants and 20 pods were assessed for shatter resistance by RIT. The lengths of a further ten pods were recorded and the weight of each pod component weighed separately after separating the valves from the beak plus septum and the seed. The number of seeds in each pod was also recorded.

Shatter resistance in DK142 and Apex in different field environments

Seeds of DK142 and Apex were sown at Long Ashton, Norwich (John Innes Research Centre) and Gent (Bayer BioScience N.V.) during the winter of 2000 and at Gent and Long Ashton in early spring of 2001. The aim was to determine the extent of variation in shatter susceptibility in populations of each line grown under different environmental conditions.

Sowing times in 2000 and subsequent glasshouse conditions were varied from site to site during November and December in order to bring plants that were grown in John Innes No. 2 compost in 7.5-cm diameter pots to the four-leaf stage during January. Plants were vernalized at ambient temperatures in a gauze house at Long Ashton, under polythene at Gent and in controlled environment rooms at 6 °C for 8 weeks at Norwich during January and February 2001. Losses occurred during winter but approximately 100 DK142 and 50 Apex were available for planting outside prior to stem extension during March at all three sites. Plants at all sites were grown 0.5 m apart in double rows at least 1 m apart. At Long Ashton, DK142 was grown in two rows and Apex in a single row of plants. At Gent, blocks of DK142 and Apex were grown within a larger planting scheme. At Norwich, plants were grown in rows of repeated sequences of five plants of DK142 and five of Apex.

Standard agronomic treatments were used at each site to prevent pests and disease throughout development. Flowering took place at all sites during May. All pods were fully ripe by early August but weather conditions delayed collection of pods for analysis until September.

In Gent and Long Ashton, second sowings of DK142 and Apex were made in February 2001. Seedlings were artificially vernalized at 6 °C for 8 weeks. In Gent plants were transplanted in the field on 14 June. Plants were grown in rows in blocks of 50 plants. Flowering during July was followed by an extended period of growth before 53 plants of DK142 were fully mature and ready for collection in October. Only 12 Apex plants were mature at this time and the remainder subsequently failed to ripen. At Long Ashton, plants were transferred to John Innes No. 2 compost in 15-cm diameter pots and set out 0.5 m apart in rows 1 m apart in a cold glasshouse on 9 May. Each line was grown in rows of nine plants that were randomized in 13 blocks. Flowering during late June and early July was followed by a period of 9–10 weeks before the pods of all plants were fully ripe in October, when 116 plants of DK142 and 58 of Apex were collected for RIT analysis.

Shatter resistance amongst populations derived from crosses between DK142 and Apex

The glasshouse-grown F_1 plants were infected by much fungal growth during the later stages of pod development and although seed production for the F_2 s was unaffected, the pods were not suitable for shatter analysis. Therefore, a cutting from each of 40 F_1 plants and from Apex plants were rooted in a propagator. These plants developed rapidly under glass, flowered and produced fully mature pods in July that were harvested and used for RIT shatter analysis.

Seeds of Apex and F_2 were sown in Norwich and Gent in December 1999. At Gent seedlings were transferred to the field where they were planted 0.5 m apart, in rows that were at least 1 m apart. Vernalization took place outside during January and February 2000 at ambient temperatures. At Norwich seedlings were pricked out into John Innes No. 2 compost, vernalized in controlled environment rooms at 6 °C for 8 weeks and then planted 0.5 m apart, in rows that were at least 1 m apart. Pods were harvested in the field as soon as possible after ripening in Gent in August and in early October in Norwich. Pods were collected from the terminal and first primary raceme of all plants and 20 pods were assessed for shatter resistance by RIT. The lengths of a further 10 pods were recorded and the weight of each pod component weighed separately after separating the valves from the beak plus septum and the seed. The number of seeds in each pod was also recorded.

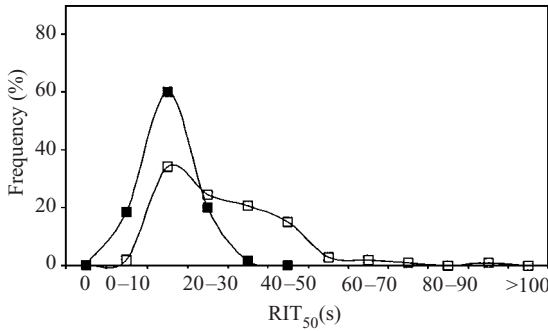


Fig. 1. Frequency distributions of shatter resistance measured by Random Impact Test (RIT₅₀) of Apex (closed squares) and DK142 (open squares) populations grown under glass in 2000.

Statistical analysis

Analyses of variance were carried out using the statistical package GENSTAT 5 (Genstat 2000) to calculate standard errors of differences between means. Where there were more than two treatments, significant differences at $P < 0.05$ were calculated in *t*-tests.

RESULTS

Range of shatter susceptibility and components of pod structure in DK142 and Apex plants grown in a glasshouse during 2000

Large differences in shatter susceptibility were recorded between populations of Apex and DK142 (Fig. 1). Although minimum shatter susceptibility (RIT₅₀) values were less than 10s in both lines and the mode was identical when using 10s class intervals, the mean RIT₅₀ for DK142 (29s) was nearly two-fold greater than for Apex (15s) and the maximum for DK142 (99s) was three-fold greater. Moreover, when class intervals of 5s were used the mode of DK142 was 5s larger than for Apex. The distribution for DK142 was skewed compared with the near normal distribution in Apex. The increased range in DK142 was accounted for by 86% of its values that were greater than the mean for Apex and 33% of the values greater than the maximum RIT₅₀ value of 32s for Apex.

Relationship between stem growth and shatter susceptibility

Stem height of Apex measured during and at the completion of extension had statistically significant correlation with RIT₅₀ values of mature pods, suggesting plant vigour has a role in shatter resistance (Table 1). For Apex, the positive relationship was established as soon as stem elongation began in early March. In contrast, the correlation with stem height was not

Table 1. Stem heights (mm) during and at the end of extension of DK142 and Apex grown under glass in 2000 and correlation (*r*) values of the variation in height with shatter resistance measured by a Random Impact Test (RIT₅₀). DK142, *n* = 108; Apex, *n* = 134

	Min	Max	Mean ± S.E.	<i>r</i> (RIT ₅₀)
DK142				
One week	12	110	40 ± 1.3	0.11
Three weeks	47	323	141 ± 4.5	0.06
Final stem height	255	1000	636 ± 13.3	0.31**
Apex				
One week	0	15	1 ± 0.3	0.21*
Three weeks	0	134	28 ± 2.2	0.39***
Final stem height	33	845	434 ± 17.1	0.48***

*, **, ***, *r* values significant at 5, 1 and 0.1%.

significant in DK142 until final heights had been established in the middle of April and then the correlation was weaker than for Apex.

Morphological differences in pod structure

The external appearance of mature pods from the two lines was distinctly different (Fig. 2). DK142 pods had thicker pedicels, pod walls and beaks than Apex. Pods of DK142 were 15% shorter than Apex and contained only half the number of seeds (Table 2). However, the valves were more than 25% heavier and the beak plus septum more than 50% heavier than Apex. Despite the heavier receptacle of DK142, the total pod weight was 23% lighter than in Apex, because its seed weight was 55% less. Indeed, the partitioning ratio of seed to receptacle in Apex was 1.7:1, whereas for DK142 the ratio was 0.6:1.

For Apex the weight of the entire pod, the weight and length of the valves and the weight of the beak plus septum correlated significantly with one another ($r = 0.65-0.91$, all significant at 0.1%). All these parameters correlated significantly and positively with RIT₅₀ values (Table 2). The correlation of the variation in seed weight and RIT₅₀ values was only just significant. Similarly for DK142, the weight of the valves correlated well with the weight of the beak and septum ($r = 0.56$, significant at 0.1%) and valve length ($r = 0.62$) and as with Apex, longer and heavier pods were more resistant ($r = 0.47-0.60$). However, seed weight did not correlate significantly with RIT₅₀ values.

As final plant height and pod characters of both lines were positively correlated with resistance, the relationship between plant height and pod characters was investigated (Table 2). Taller plants of both lines had significantly heavier pods. For Apex, heavier pods were a result solely of heavier valves, whereas all components of pods from tall plants of DK142 were

Table 2. Descriptive statistics of mature pods of DK142 and Apex grown under glass in 2000 and correlation (r) values of the variation in pod parameters with final plant height and shatter resistance measured by a Random Impact Test (RIT_{50}). Values in parentheses are weight of organ as percentage of the total pod weight

	Line	Min	Max	Mean	S.E.D. (179 D.F.)	r (RIT_{50})	r (height)
Weight per pod (mg)							
Whole pod	DK142	86	232	143 (100)		0.52***	0.41***
	Apex	122	288	185 (100)	4.2	0.43***	0.39***
Valves	DK142	35	114	68 (47)		0.60***	0.34***
	Apex	30	90	53 (29)	1.9	0.49***	0.61***
Beak + septum	DK142	14	41	24 (17)		0.56***	0.26**
	Apex	10	27	16 (9)	0.6	0.50***	0.13
Seed	DK142	6	96	52 (36)		0.15	0.31***
	Apex	69	172	116 (63)	2.5	0.20*	0.09
No. of seeds per pod	DK142	2	18	11		0.13	0.35***
	Apex	12	29	23	0.5	0.16	-0.13
Pod length (mm)	DK142	35	67	52		0.47***	0.37***
	Apex	50	69	61	0.7	0.31**	0.26**

*, **, ***, r values significant at 5, 1 and 0.1%.



Fig. 2. Typical pods from Apex (left) and DK142 (right).

heavier and in contrast with Apex the number and weight of seed was greater.

Effects of removal by pruning of all but terminal and first lateral racemes on shatter susceptibility, pod structure and size

In the subset of 14 plants that had been pruned 5 weeks after anthesis, mature pods were approximately 50%

more resistant than on the unpruned, control plants in both DK142 and Apex (Table 3).

Although fractionally longer, pods from pruned Apex plants weighed 39% more than pods from unpruned plants. Pods from pruned DK142 plants were not longer than from unpruned plants but were also significantly heavier by 20%. In both lines, pruning increased the weight of the pod receptacle more than the weight of the seed. In Apex, receptacle and seed

Table 3. Effect of removal of all but the terminal and first lateral raceme on components of pod structure and shatter resistance measured by a Random Impact Test (RIT₅₀). Plants were grown under glass in 2000. Values in parentheses are weight of organ as percentage of the total pod weight

	Apex		S.E.D. (86 D.F.)	DK142		S.E.D. (86 D.F.)
	Unpruned	Pruned		Unpruned	Pruned	
RIT ₅₀	14	22	1.5	27	41	3.7
Mature pods						
Weight per pod (mg)	176 (100)	245 (100)	5.7	141 (100)	168 (100)	8.1
Valves	50 (28)	75 (30)	2.6	66 (47)	85 (51)	4.0
Beak + septum	16 (9)	21 (9)	0.7	23 (16)	27 (16)	1.6
Seed	110 (63)	149 (61)	3.8	52 (37)	56 (34)	4.9
No. of seeds per pod	23	26	0.9	11	11	1.0
Pod length (mm)	61	66	0.9	51	54	1.7

weight were increased by 45 and 35%, whilst in DK142 the increases were 26 and 7%. In Apex the weight of seed was increased as a consequence of more seed (Table 3). The extra pod receptacle weight was mainly a result of thicker valve walls, which in pods from pruned plants were heavier per unit length by 39% in Apex and 22% in DK142. Septum weight per unit length was also heavier by 23% and 10% in pods from pruned Apex and DK142 respectively.

Variation in shatter susceptibility within field grown populations of DK142 and Apex: site to site comparisons for populations planted in the field in March 2001 ('early-sown')

For each of the lines the range of shatter resistance values (RIT₅₀) was similar at all sites (Fig. 3). In populations of Apex, the distribution at all sites was similar with an overall mean of 18 ± 0.7s. The range of resistance was narrow (<10–40s) and was normally distributed about a modal class 10–20s. The mean RIT values for DK142 were on average three times greater than RIT₅₀ values for Apex and the range of shatter values was much wider (<10s–169s) with the most frequent scores between 25–40s. The mean values were statistically similar (P=0.23) at Long Ashton (51 ± 3.6s) and Gent (47 ± 3.2s) but a few high scoring values recorded in samples collected at Norwich increased the mean at this site to 73s. The distributions were broadly similar at each site and skewed compared with Apex (Fig. 3) with over 90% of RIT₅₀ values from each of the three DK142 populations greater than the Apex mean and between 52 and 73% of RIT₅₀ values greater than the Apex maximum.

Populations planted in June 2001 ('late-sown')

The range and mean shatter resistance values for the 'late-sown' field grown population of DK142 at

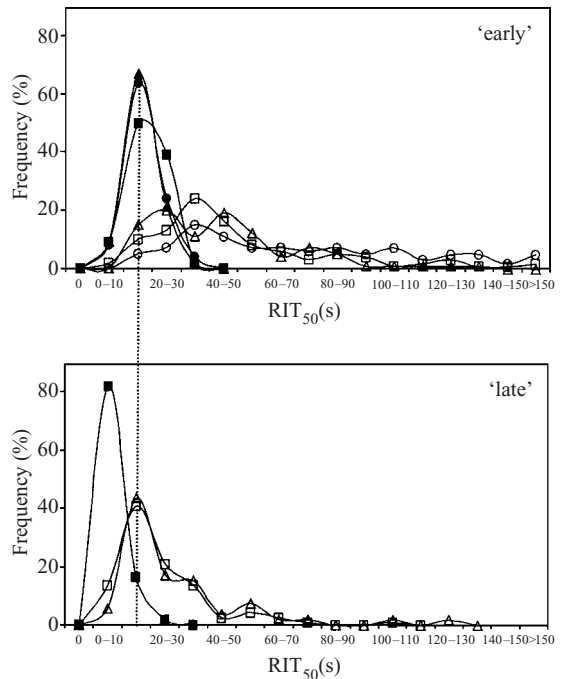


Fig. 3. Frequency distributions of shatter resistance measured by Random Impact Test (RIT₅₀) of Apex (closed symbols) and DK142 (open symbols) populations grown during 2001 from 'early'- and 'late'-sowings at Long Ashton (squares), Norwich (circles) and Gent (triangles). Note the increased susceptibility of 'late'-sown populations.

Gent (mean = 28 ± 3.2s; range <10–130s) were similar to those for the 'late-sown' population grown at Long Ashton under glass (mean = 24 ± 1.5s; range <10–100s). However, the mean shatter resistance values were approximately half those for the 'early-sown' populations (P<0.001) and the ranges were narrower. Moreover, for both 'late-sown' DK142

Table 4. Characteristics of fully mature pods from F_1 plants ($n=40$) and correlation (r) values of shatter resistance measured by a Random Impact Test (RIT_{50}) with the variation in pod parameters. Values in parentheses are weight of organ as percentage of the total pod weight

	Min	Max	Mean \pm s.e.	r (RIT_{50})
RIT_{50}	<10	36	16.9 \pm 0.8	
Weight per pod (mg)				
Valves	29	64	50 \pm 1.3 (38)	0.45***
Septum	10	16	14 \pm 0.2 (10)	0.40***
Seed	43	94	67 \pm 2.2 (51)	0.18
No. of seeds per pod	9	22	15 \pm 0.5	0.04
Pod length (mm)	44	120	62 \pm 1.9	0.21*

*, **, ***, r values significant at 5, 1 and 0.1%.

Table 5. Characteristics of fully mature pods of F_2 ($n=197$) plants grown in the field at Norwich and Gent in 2000 (combined data) and correlation (r) values of shatter resistance measured by a Random Impact Test (RIT_{50}) with the variation in F_2 pod parameters. Shatter resistance values for Apex plants ($n=93$) grown in the field are also presented as a comparison. Values in parentheses are weight of organ as percentage of the total pod weight

	Min	Max	Mean \pm s.e.	r (RIT_{50})
RIT_{50} for F_2	<10	324	41 \pm 2.7	—
RIT_{50} for Apex	<10	35	18 \pm 0.6	—
Weight per pod (mg) F_2				
Whole pod			204 \pm 4.5 (100)	
Valves	30	138	78 \pm 1.6 (38)	0.58***
Beak + Septum	10	54	26 \pm 0.6 (12)	0.50***
Seed	40	158	100 \pm 2.6 (49)	0.29**
No. of seeds per pod	6	27	17 \pm 0.5	0.20**
Pod length (mm)	36	86	61 \pm 0.6	0.26**

*, **, ***, r values significant at 5, 1 and 0.1%.

populations the mode was lower by 20s. Indeed, the frequency class range for the mode was shifted into the range typically occupied by 'early-sown' Apex.

The only population of 'late-sown' Apex that could be harvested was that grown under glass at Long Ashton. The range of resistance was much narrower (<10–22s) than for 'early-sown' populations and was normally distributed about a modal class of 0–10s which was lower than 'early-sown' populations by 10s. The mean value of 8s was less than half those for the 'early-sown' populations ($P < 0.001$).

Clearly sowing late, planting out in June and harvesting in September caused an increase in shatter

susceptibility compared with populations sown early, planted out in March and harvested in July. However, as seen in comparisons of 'early-sown' populations there was little site-to-site variation in the 'late-sown' populations and within each sowing period mean values were consistently three times greater for DK142 than for Apex.

Shatter susceptibility and pod characters in derived lines

RIT_{50} values in all F_1 plants grown from cuttings in a glasshouse (Table 4) were similar to those recorded for pods from cuttings of the parent line Apex (mean RIT_{50} , 13s; range <10–25s). Pod length, valve and beak and septum weight of F_1 pods were all similar to those of Apex (cf. Table 2; data for pod parameters from Apex cuttings were similar to those from plants grown from seed) and there were significant and positive correlations of the length and weight of the components of the pod receptacle with RIT_{50} . As with both parents, the mean weight and number of seeds per pod did not correlate strongly with RIT_{50} values and both were greater than for DK142, though less than Apex.

In the F_2 populations grown in the field at Norwich and Gent in 2000, the overall mean RIT_{50} was 127% greater than for Apex (Table 5). Although minimum RIT_{50} values in the F_2 populations and in Apex were less than 10s, the maximum for the F_2 s was almost 10 times greater than for Apex.

The distribution of the F_2 values was similar to the generally more resistant parent (DK142) in that the distribution was wide and extended from <10 to >100s (Fig. 4). Resistance values for 98% of the plants were found in the continuous part of the range which extended to 110s, whilst 2% of the plants (four plants) had much higher RIT scores measured at 173, 193, 300 and 323s.

Data for pod parameters for Apex were similar to those given in Table 2 and are not shown. In the F_2 population the range of values for the pod receptacle characters spanned the combined range of the two parent lines (cf. Tables 2 and 5). On average, pods tended to be heavier than both parents and F_1 pods, a result of the interaction of inheriting pods which on average were as long as those from the commercial parent, but which also had the heavier valve weight per unit length of the near-synthetic parent (mean valve weight per unit length for Apex, 0.82; DK142, 1.27; F_2 , 1.29). Correlations of the resistance scores with the various parameters confirmed the linkage of the length and particularly the weight of the pod receptacle with resistance (Table 5). This was also seen in the F_1 population where the amount of variation in resistance values and pod dimensions was very much smaller.

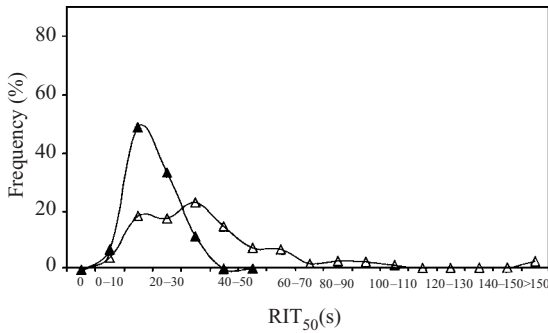


Fig. 4. Frequency distributions of shatter resistance measured by Random Impact Test (RIT_{50}) of Apex (closed symbols) and F_2 (open symbols) populations grown in the field in 2000.

DISCUSSION

Comparisons of the shatter distribution patterns for each of the DK142 and Apex populations identified a lack of environmental influence on shatter resistance mediated through site-to-site variation. Different geographical locations, growing conditions and growing year had little or no effect and for populations of Apex, the distribution and mean of shatter resistance in pot-grown plants under glass in 2000 was almost identical to those recorded in field-grown populations of 2000 and 'early-sown' 2001. The time of sowing was the only factor that influenced shatter susceptibility within populations of both lines. In 2001, the three 'early-sown' populations of both DK142 and Apex had similar shatter statistics within a line, with the mean RIT_{50} score for each line higher and the range wider than populations sown 'late' in 2001. For DK142 the distribution and mean of shatter resistance in pot-grown plants under glass in 2000 at Long Ashton was more similar to those recorded in the 'late-sown' rather than the 'early-sown' field-grown populations of 2001. Pot-grown plants were from sowings in December, which was relatively late compared with normal sowing times at Long Ashton and appears to have influenced the shatter statistics of DK142, although not Apex, in a similar manner to sowing 'late' in the field.

Plants set out in the field in June from 'late' sowings would have accumulated lower assimilate levels prior to flowering compared with the 'early-sown' plants. Indeed, the average stem height of 'early-sown' field-grown DK142 plants at Long Ashton was 634 mm compared with 501 mm for 'late-sown' plants. As crop height is clearly associated with total biomass, reduced plant weights in 'late-sown' plants may be relevant to the reduced resistance of DK142 planted out in June. Time of sowing did not affect Apex plant height, although the stems were thinner in the 'late-sown' population (data not shown).

Within individual populations of DK142 and Apex, plant to plant differences in height also correlated with shatter resistance (Table 1), with taller, more vigorous plants producing longer, heavier and more shatter-resistant pods (Table 2). This positive relationship between increasing stem height and shatter resistance has been previously reported by Morgan *et al.* (2000). For Apex, the positive relationship of resistance with stem height was clear from the first week of stem elongation and may have been determined even earlier, whilst for DK142 the relationship was significant only when growth had finished. For both lines, the weight of the receptacle was heavier from taller plants, but for Apex, plant height did not correlate with seed weight per pod nor seed number. Thus, the partitioning of dry matter between the pods and their contents in tall Apex plants was similar to that found in DK142. Partitioning of assimilates to seeds appears to have been determined early in pod development whilst partitioning to the rest of the pod depended on availability of resources throughout development. Thus shatter resistance values may also be influenced throughout pod development.

The very large difference in shatter resistance values of Apex and DK142 strongly indicates a genetic component, but the importance of stem height and, therefore, plant vigour and biomass is particularly relevant to agronomic management. Thus, once the introgression of the DK142 shatter resistant trait into breeding lines is accomplished successfully, appropriate husbandry requirements may also need to be reassessed as recoverable yields may be further augmented by improvements in the nutritional status of the crop. This may be achieved by increased inputs, but improved individual plant biomass can also result from reduced sowing density and would promote shatter resistance via the formation of heavier pod receptacles. Moreover, in contrast with Apex, the seed weight from pods from taller, more vigorous plants of DK142 was increased as a result of increased seed number, thus improved nutritional status may further amplify the yield.

The distribution pattern of RIT_{50} values in the F_1 population was more similar to that found in Apex than to that of DK142 suggesting shatter resistance is a recessive trait, whereas the distribution pattern recorded in F_2 populations was similar to that recorded for DK142. This confirms the heritability of this trait and suggests that the genes and characters associated with increased shatter resistance are present in the populations of both the synthetic and the derived F_2 plants.

The most marked difference between pods from the two parents was the weight and size of the receptacle and although DK142 pods were much shorter, they were heavier than those of Apex. Within line variation of these characters in the parent, F_1 and F_2

populations was monitored to determine which particular parameters determined shatter resistance. In all populations, including those with all or the majority of plants falling within a small range of resistance, dimensions of the pod receptacle correlated positively with shatter. Variation in valve and beak plus septum weight produced the most significant correlations, closely followed by valve length and thus septum length. As both parents can contribute positive characters, i.e. long pods from Apex and pods with a high weight/unit length from DK142, the range of resistance in segregating populations might have expected to exceed that found in the parents. This was the case in four plants in the F_2 population, which had RIT scores higher than the range seen in DK142. These four plants had pods longer than the average DK142 pod, with three as long or longer than the average Apex pod and in each case, valve weight per unit length (1.5–2.11) was close to or equalled the maximum values found in the DK142 population.

Although the receptacle weight of the DK142 pods was heavier than in the longer Apex pod, the seed weight was 50% lighter due to 50% fewer seeds and contrasted strongly with Apex where the weight distribution was clearly partitioned towards the contents rather than the receptacle. The ranges of the seed and receptacle weights in the F_2 population was similar to the combined ranges of the two parents but on average, mean receptacle weight was more closely aligned to DK142 and seed weight to Apex despite seed number being midway between the two parents. Thus, compared with the two parent lines, the F_2 population showed improved resistance compared with Apex and improved seed set over DK142. Indeed, seed weight per pod was only 10% less than for Apex and although this is agronomically undesirable, the positive correlations of seed number and seed weight with RIT₅₀ values in the F_2 population suggests that restoring normal fertility should be compatible with retaining the enhanced shatter resistance.

The parental line difference in seed number per pod did not appear to result from differences in ovule numbers, which were similar in both lines in response to self- or cross-pollinations by hand (data not shown). Although pollen production was delayed and reduced in DK142, lower seed number in this line may be more connected with partial infertility inherited from its resynthesized parent. We have also noted that line DK142 has a tendency to set fewer pods. Reduced seed and/or pod set in this line may be responsible for increased partitioning to the receptacle that may be a causal factor helping to determine increases in shatter resistance. However, within line DK142 the variation in number and weight of seed per pod did not correlate significantly with resistance so pod numbers per plant may be more relevant.

It was not known whether the heavier pod of DK142 was a cause or consequence of lower pod density or whether it was determined genetically by factors other than fertility. Removal by pruning of lower racemes and the consequent reduction in pod numbers enabled a comparison to be made of the effects of pod density on shatter susceptibility. Pruning increased the weight of the remaining pods by nearly 40 and 20% for Apex and DK142 respectively, with the increase in weight biased towards the seed receptacle. The RIT₅₀ values in pods from pruned plants were on average 50% more resistant than pods from unpruned plants. As pods from pruned Apex plants were still more susceptible than those from DK142 with a similar number of pods, the results support the view that increased shatter resistance in DK142 pods is genetically determined. However, pruning enhanced resistance in both lines, suggesting competition for resources within the plant also helps to determine the final level of resistance. The genetic and competitive influences on shatter status may have different mechanisms, the resolution of which requires anatomical and perhaps biochemical study.

In all populations, the weight of the septum and of the valves each correlated well with resistance. These two components of the pod are joined at a DZ which is located in sutures that extend on each side and along the length of the two valves. The DZ consists of rows of two or three simple, thin-walled cells that are separated from the valve by heavily thickened cells and which join with the heavily vascularized replum of the septum. During the later stages of pod senescence, approximately 7 weeks after anthesis the thin-walled cells separate. The valves separate when impacts with other pods or with the combine break residual adhesions. Heavy receptacles may cushion DZ cells from impact, although if this were so, a strong positive correlation with pod wall thickness and resistance would be expected. As yet there is no published evidence that this is the case and it may be more likely that the weight of these organs affects resistance because they determine the size of the DZ. Clearly, valve length determines the length of the DZ and in all populations this parameter correlated well with resistance. However, although DK142 was more resistant, average pod length was greater in Apex than in the DK142. Correlations between the valve and septum weights with RIT₅₀ in the parent lines and the F_2 populations were also high and the more resistant parent line produced pods with heavier valves and septum despite their shorter length. We believe that pods with a heavier receptacle have a wider and thus more resistant DZ and that in addition, the DZ of DK142 has different separating properties compared with Apex. Investigations of the differences in DZ size as well as its structure in relation to pod wall architecture are underway in our laboratory.

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