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Recurring Off-types in Lettuce: Their Significance in Plant Breeding and Seed Production

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Summary. The lettuce cv. Valentine regularly produces non-heading off-type plants at the relatively high frequency of 4×10^{-3}. They result from mutation to the dominant condition. A similar propensity has been reported twice before in lettuce; it is highly heritable.

The propensity to produce off-types was not present in the parents of Valentine so it must have arisen during the pedigree breeding programme. Closely related cultivars do not carry the character but it was subsequently transmitted to Dandie a new cultivar of which Valentine was one of the parents. Allelism tests to relate Valentine off-types to those in crisphead cultivars were not successful.

The elimination of off-types in breeding programmes is discussed. Contrary to the findings in day-neutral crisphead cultivars, mutant phenotypes in long-day butterhead cultivars have no selective advantage for seed production. Consequently, strict roguing ensures adequate varietal stability.

Key words: Lettuce - Crisphead Cultivars Off-types Instability

Introduction

The commercial exploitation of cultivars produced by State-aided Institutes in the United Kingdom is the responsibility of the National Seed Development Organisation Ltd., (NSDO). Breeders' seed is maintained by the Institutes; basic seed is produced by the NSDO. Samples of basic seed are always checked for performance against breeders' seed before the bulk is passed to the seed trade for further multiplication and sale. During the final multiplication of two glasshouse lettuce varieties, Valentine and Dandie, a recurring and less-well-hearted off-type was found in both breeders' and basic seed.

Non-heading mutants have previously been described in the yellow-leaved long-day butterhead cultivar Empereur (syn: Kaiser Treib and Kaiser driv) by Bremer and Grana (1935) and by Lindqvist (1960) and also in the day-neutral crisphead cultivars Cornell 456 (Pearson 1956; Pearson 1968) and Great Lakes (Pearson 1968). This paper describes the off-type found in Valentine and relates it to the previously reported off-types. Its importance relative to future breeding programmes and to seed stock maintenance is discussed.

Results

Description and occurrence of the off-type in Valentine

When compared with Valentine, its off-type is larger leaved, later maturing and taller. The off-type also has smoother, slightly darker green leaves (Fig. 1). In the close plant spacings used for protected crops these off-types form loose and late maturing heads rather than

Fig. 1. Valentine off-type - one off-type surrounded by true types

Table 1. Lettuce Off-types Valentine Breeders' Seed

Year	1966	1967	1968	1969	Total
Plants	840	637	720	2105	4302
Off-types	6	2	2	7	17
%	0.71	0.31	0.28	0.30	0.40

Table 2. Lettuce Off-types Valentine Basic Seed

Year	1967	1968	1969	Total
Plants	320	1260	1512	3092
Off-types	2	11	8	21
%	0.63	0.87	0.53	0.68

open rosettes. They are marketable but need to be packed separately. The overall percentage of off-types recorded in breeders' seed in the years 1966 to 1969 was 0.4 percent with some year to year variation (Table 1). The off-type occurred at random within the total sample of 86 single plant progenies grown and its form was constant.

The number and frequency of off-types recovered from basic seed in the years 1967 to 1969 are shown in Table 2. The overall frequency was 0.68 percent. Half of the plants used to produce the basic seed tested in 1968 were from an insect-proofed glasshouse and and the other half from an open-ventilated glasshouse. They produced six and five off-types respectively; all were similar to each other and to those found in other years.

Characterisation of the off-type

Table 3 summarises the kinds of off-types found in crop plants. Because of its regular occurrence, uniform appearance from generation to generation and its relatively constant low frequency, the described off-type most closely resembles a condition induced by mutation although its frequency at 4×10^{-3} is high compared with the average rate of 2×10^{-6}.

Ten off-type plants from breeders' seed of Valentine were self-pollinated and seeded. Within the segregating M_2 generation families it was possible to recognise three kinds of plant; Valentine-like; off-types; and a third category with a much more rosetted habit and even looser heads than the original off-type. This suggested that the off-type results from the mutation of a single gene to the incompletely dominant condition. Three families, B4896, B4895 and B4897, were chosen for further study.

Attempts were made, at various stages of growth, to classify the plants of these families into the three postulated classes on visual appearance. A method of identifying the off-type at an early stage of growth would be useful for roguing seed crops so the length and breadth of the second true leaves was also recorded. All plants were progeny tested and it was then possible to classify them accurately into the three genotypes: +/+ (true Valentine), OT/+ (heterozygous off-type) and OT/OT (homozygous off-type). As there is no proven relationship with previously reported off-types, the symbol OT is used here for convenience, it is not proposed as a gene symbol. The summarised data are given in Table 4. The segregations show good agreement with a 1:2:1 ratio and the results are in

Table 3. Off-types In Crop Plants

Cause	Occurrence	First Generation		Behaviour in later generations
		Appearance	Frequency	
Contamination	Sporadic	Uniform*	Variable	Breed true
Cross pollination /Recombination+	Sporadic	Uniform*	Variable	Show polygenic recombination
Mutation	Regular	Uniform	Constant low	Show monogenic segregation

* Assuming one uniform source of seed or pollen contamination
\+ Resulting from cross-pollination in any earlier generation

Table 4. Valentine Off-types. Progeny test of M_2 families

Family	+/+	OT/+	OT/OT	Total	χ^2	p
B4896	23	32	19	74	1.785	0.5-0.3
B4895	21	42	12	75	3.240	0.2-0.1
B4897	11	18	10	39	0.281	0.9-0.8
Deviation	55	92	41	188	2.171	0.5-0.3
			Heterogeneity		3.135	0.7-0.5

agreement for the three families. This confirms that the off-type originates by mutation to the incompletely dominant condition.

In Fig.2 the visual assessment of plants in family B4896 at the mature stage is compared with their known genotypes from progeny testing. It shows that twenty of the thirty-four plants were correctly classified by appearance; the fourteen misclassifications resulted mainly from incorrect identification of the heterozygotes. Similarly, Fig.3 shows leaf measurements for plants of known genotype; leaves of +/+ plants tend to be relatively short and broad, those of OT/OT long and narrow, with the heterozygotes intermediate. Similar results were obtained with the other two families. The material was favourable because of its uniform genetic background, and the plants were grown in the relatively uniform environment of the glasshouse, but even so, the scoring was not infallible. Reliable classification can only be achieved by progeny testing.

The glasshouse lettuce cultivars grown for crop production in the autumn, winter and spring period have a long-day photoperiodic response. Seed crops

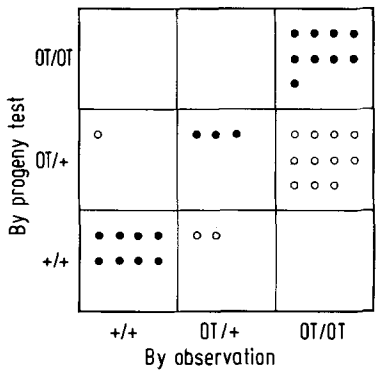

Fig.2. Valentine off-type. Whole plant classification. M_2 generation

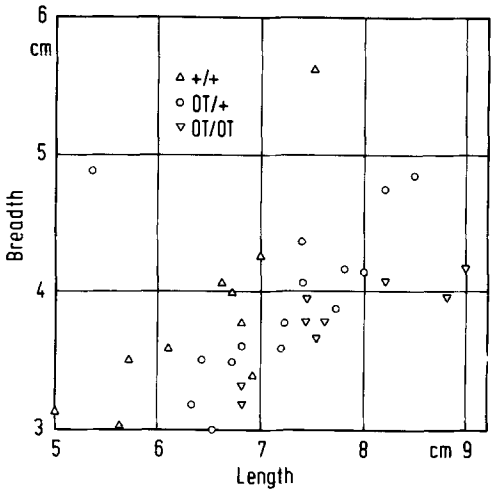

Fig.3. Valentine off-type. Leaf morphology in M_2 generation

are often grown during the summer months when these cultivars proceed straight to flowering without forming a head. Under such conditions it is impossible to identify heading characteristics and consequently the off-type cannot be rogued out. It is therefore important to know the seed-producing potential of the three genotypes in order to determine whether the heterozygous or homozygous off-type plants are at a selective advantage.

Plants of the three families were grown on to produce seed; all survived and their yields are shown in Table 5. There were no significant differences between the genotypes.

The off-type in cultivars related to Valentine

The pedigrees of Valentine and related cultivars have been published (Maxon Smith 1972) and are summar-

Table 5. Valentine Off-types. Seed Yields (g per plant)

Family	+/+	OT/+	OT/OT	Mean
B4896	2.89	3.95	3.73	3.52
B4895	3.58	2.95	1.55	2.69
B4897	3.36	3.63	4.27	3.75
Mean	3.28	3.51	3.18	3.32

Based on 108 plants. No significant differences

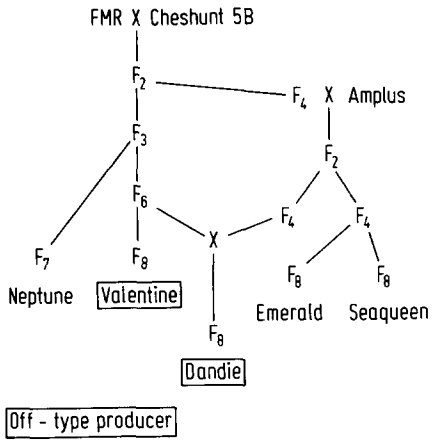

Fig.4. Pedigrees of Valentine and related cultivars

ised in Fig.4. Six of these cultivars have been surveyed for off-type production; the results are shown in Table 6. Whenever possible at least 670 plants per cultivar were grown; this being the number calculated to include at least one off-type plant in 19 out of 20 trials when the mutation rate is 0.4 per cent.

The original cross between Forcing Mildew Resisting and Cheshunt 5B was made in 1956 using selected plants grown from commercial seed. Stocks of Cheshunt 5B then available were highly variable and also virus infected. Single plant selection over several years at the GCRI led to an improved, uniform, virus-free type which was released as Southdown 5B (Maxon Smith 1972). Both Cheshunt 5B and Southdown 5B were used in this survey but the results can be considered together since neither produced off-types, and single plant selection would be unlikely to eliminate the ability to produce off-types should it have existed in the original cultivar. It was also found that the black seeded Forcing Mildew Resisting and Resistant Early French Frame which are usually listed as synonyms of Gotte à forcer à graine noire (Rodenburg et al. 1960) were identical. The commercial seed stocks obtained in 1971 and used for this study contained contaminant plants which were large and leafy and sometimes white seeded. Progenies derived from these were quite mixed which suggests that the source of these off-types was pollen contamination in a previous seed crop, see Table 3. Five true-to-type plants of Forcing Mildew Resisting were seeded and their progenies grown the following year, none of the 1,430 plants were off-type. Valentine produced 0.43 per cent off-types; the very closely related Neptune had none.

Table 6. Off-types In Long-day Butterhead Cultivars

Cultivar	Plants	Off-types
Cheshunt 5B	560	0
Southdown 5B	700	0
Forcing Mildew Resisting	560	17*
Resistant Early French Frame	650	13*
Valentine	700	3
Neptune	675	0
Forcing Mildew Resisting (reselected, true to type)	1430	0

* Due to cross-pollination, see text

Evidence on the other varieties in Fig.4 is less formal but equally convincing. Amplus is a variety of Dutch origin which has never been observed or reported to produce off-types. In the production of breeders and basic seed of Emerald and Seaqueen, during which large numbers of plants were carefully screened, no evidence of off-types has been found. Conversely Dandie, a new cultivar which had Valentine as one of its parents, does produce off-types. They appear to be similar to and occur at the same frequency as Valentine off-types.

Relationship to other reported off-types in lettuce

As outlined in the introduction rosetting or non-heading mutants in lettuce have been recorded previously. Earlier descriptions completely fit that for the Valentine off-type. All originate as high frequency mutations to the dominant condition and segregate 1:2:1 on progeny testing.

It has not been possible to obtain seed of Empereur, the first reported source, but five day-neutral cultivars have been assessed for off-types in outdoor summer crops (Table 7). Cornell 456 was the main cultivar studied by Pearson (1956, 1968), who also traced the off-type to one of its parents, Brittle Ice, which is a synonym of Iceberg. Histon Krispie is regarded as a synonym of Cornell 456.

The rather high frequencies of off-types found in Iceberg and Cornell 456 suggest that the seed was from incompletely rogued stocks. This was confirm-

Table 7. Off-types in Day-neutral Cultivars

Cultivar	Plants	Off-types	%	Off-type progenies	
				No.grown	Status
Cornell 456	732	20	2.73	5	OT/OT
Iceberg	732	14	1.91	4	OT/OT
Histon Krispie	732	3	0.41	3	Segregated
Avoncrisp	732	2	0.27	2	OT/OT
Avondefiance	732	0	0	-	-

ed by the progeny testing which revealed that some of the plants had the OT/OT genotype. Conversely, Histon Krispie appeared to be a well-rogued stock with 0.4 per cent off-types. Their progenies segregated, indicating that the mutation had newly occurred. The occurrence of off-types in Avoncrisp was surprising since they had not been previously reported in this cultivar. The variety Avondefiance which is classed as a butterhead was included in the test since it is related to Avoncrisp but it did not produce off-types.

Crosses were made between the normal and homozygous off-type versions of Valentine and Cornell 456, and F_2 seed was produced. The parental types and the F_2 families were grown in winter glasshouse and early summer field trials but it was not possible to carry out reliable classification into headed or rosetted types. Forty plants with seemingly definite heading or rosetting habit were seeded but their progenies revealed that in nearly half of the cases the parent plant classification had been incorrect. Similary, 140 plants seeded at random from the F_2 failed to conform to any predictable pattern because of the difficulty of accurately classifying their progenies. Plants with the long-day response bolt readily as the days lengthen, thereby obscuring their hearting characteristics. Day-neutral types form very loose heads when grown under glass during short days and this also masks any hearting tendencies. It is therefore not surprising that the cross between a long-day butterhead, Valentine, and a day-neutral crisphead, Cornell 456, failed to reveal whether the genes in the two cultivars are allelic.

Significance of the off-type in plant breeding programmes

Simplified pedigrees of some of the crisphead cultivars are shown in Fig.5. The data for Great Lakes and Cornell 456 are from Bohn and Whitaker (1951) and for Oswego, Fulton and Minetto from Raleigh and Minotti (1967). Cultivars known to produce off-types are Brittle Ice and Cornell (Imperial) 456. Cultivars reported to produce off-types include Great Lakes (Pearson 1968) and Oswego, Fulton and Minetto (Raleigh and Minotti 1967). The Great Lakes parental material produces off-types as reported by Pearson (1968) but in the absence of any published reports it is impossible to know which of the Great Lakes numbered selections also produce them. Raleigh and Minotti (1967) when discussing the presence of off-types in Oswego, Fulton and Minetto also described Great Lakes 659 but made no reference to off-types in this cultivar.

In the pedigrees of Valentine and Dandie, Fig.4, it appears that the propensity to produce the off-type arose during the selection of Valentine and was also

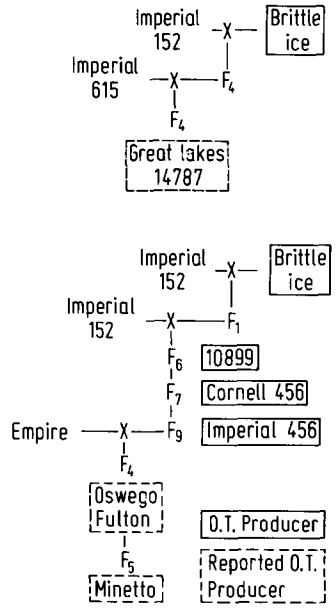

Fig.5. Pedigrees of Crisphead cultivars. (1) Known or reported off-type producers

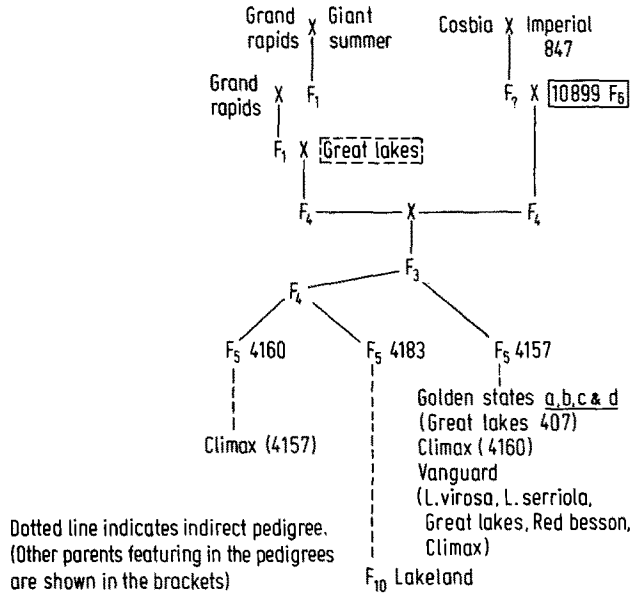

Fig.6. Pedigrees of Crisphead cultivars (2)

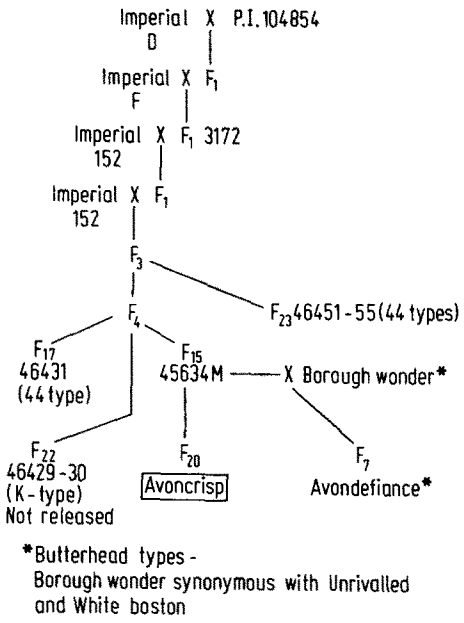

Fig.7. Pedigrees of Crisphead cultivars. (3) Cultivars with *Bremia* resistance from P.I. 104854

transmitted to Dandie. Taking the known evidence from this case and from the Cornell 456/Oswego pedigrees it seems that the off-type producing propensity once established is highly heritable.

Outline pedigrees of the varieties Climax, Lakeland, Golden States A, B, C and D and Vanguard are shown in Fig.6. They are summarised from Thompson and Ryder (1961) where full details are presented. Two known off-type producers, Great Lakes and 10899, from the Cornell 456 pedigree, feature in their parentage. There is consequently a strong possibility that some or all of these varieties produce off-types.

Further pedigrees of crisphead cultivars, Fig.7 are adapted from Whitaker et al. (1958) and the section on Avoncrisp and Avondefiance from Watts(1955). The appearance of off-types in Avoncrisp was not expected since none of its immediate ancestors had previously been reported to produce them. Their occurrence in Avoncrisp has since been reported by workers at the National Vegetable Research Station (Dixon and Lewthwaite 1972). They also reported that "Similar rogues (to the Cornell type) with similar segregational behaviour have been found in cv. Webb's Wonderful and the problem may be more general than supposed".

Indeed it may be very much more general - Fig.8 shows outline pedigrees for the early Imperial varieties based on Jagger et al. (1941). The cultivars Imperial 2, 3 and 6 were selected for brown blight resistance from New York Special and the lines 69, AA and B were similarly selected from New York. The cross New York × Blonde de Chavigne which gave rise to Imperial 13 was made in order to incorporate the brown blight resistance of Blonde de Chavigne into the crisphead type. The pedigree of Imperial 17 is from Bohn and Whitaker (1951). As can be seen from Fig.9 the subsequent Imperial types, which in-

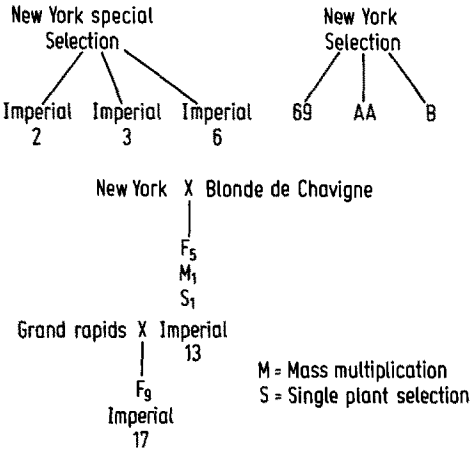

Fig.8. Pedigrees of Crisphead cultivars. (4) The early Imperial selections

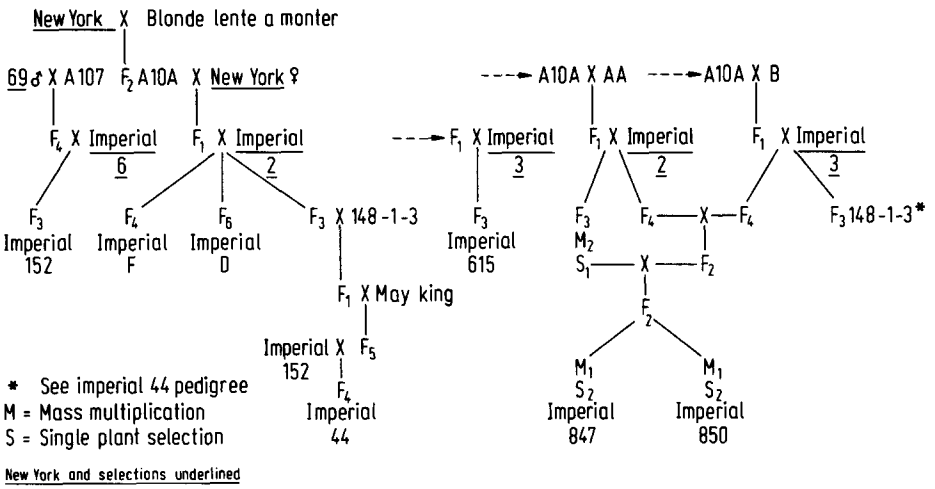

Fig.9. Pedigrees of Crisphead cultivars. (5) Later Imperial types

corporate mildew resistance from Blonde lente à monter, all feature New York or one of its derivatives in their pedigrees. New York is synonymous with Webb's Wonderful (syn. Wonderful) (Jagger et al. 1941, p1; Rodenburg et al. 1960; Watts 1955) so it is possible that some of the Imperial types produce off-types. Imperials D, F and 152 featured in the pedigree of Avoncrisp and it is from these that the off-type producing propensity of the latter may have been inherited.

Many modern crispheads have similar pedigrees to that of Avoncrisp being partly based on *Lactuca serriola* PI 91532 (now known as PI 104854) because of its race-specific mildew resistance. Thus, in addition to the 44 type selections from the same pedigree as Avoncrisp, the cross Imperial 152 × 3172 selected to F_{14} and beyond gave the Imperial D-type mildew resistant lines 46140, 46255, 44418-9 and 46450. A similar backcross series but with Grand Rapids, Imperial 847 and Imperial 850 as additional parents was selected to F_3, crossed with Cosberg and selected to F_7 and beyond to give the 325 types (Whitaker et al. 1958). Further crossing and selection of these mildew resistant lines led to the more recent crisphead cultivars such as Calmar (Welch et al. 1965). Because of the high heritability of the off-type producing propensity and its possible transmission from New York to Avoncrisp many of the modern crisphead cultivars must be regarded as potential off-type producers.

That the off-type producing characteristic is not always transmitted through the germ line is shown by cultivars closely related to Valentine which do not carry it, and by its absence from Avondefiance which is related to Avoncrisp. The appearance of the character in Valentine although it is not present in the parent varieties shows that the change from stability to mutability can arise anew.

The difficulties of identifying off-types and the necessity for confirmatory progeny tests have been discussed. These magnify the problem of selecting against the production of off-types in the early, widely segregating generations of a pedigree breeding programme. Even when the more uniform advanced generations are reached, it is necessary to screen at least 670 plants per family in order to have a good chance of detecting an off-type producing line so that the total number of plants which need to be grown in the programme is much larger. The variable environment of field crops further magnifies these difficulties.

Unfortunately it was necessary to terminate the work on day-neutral crisphead types but a more detailed survey of off-type production in these cultivars is desirable. If such types are to be used in breeding programmes attention should be given to the off-type problem before commencing.

Crisphead cultivars are being used in programmes to modify the texture of glasshouse cultivars. Valentine and Dandie are, because of their good win-

ter growth characteristics, likely to feature as parents in future breeding programmes. Consequently, there is a risk that off-type production may become widespread in glasshouse cultivars unless positive selection against this character is practised.

Off-types and seed production

Off-type production in Valentine is 0.4 per cent. Pearson (1956) gave a frequency for Cornell 456 of about one per cent although in a later paper (1968) he quoted 0.1 per cent. Off-type production in Histon Krispie which, as discussed earlier, can be regarded as a fully rogued stock of Cornell 456 was 0.43 per cent. A reasonable estimate of the rate of off-type production appears to be 0.4 per cent.

Pearson (loc. cit.) reported a three-to-eight fold survival advantage for off-type compared with normal when the densely headed crisphead varieties are grown for seed. The present study has shown no such advantage in the case of long-day butterhead types.

If seed crops of long-day lettuce cultivars are grown during the summer months, off-type detection and removal is impossible because all plants pass directly from the rosette to the flowering stage. Fig. 10 shows the theoretical build-up per generation for each genotype when the mutation rate is 0.4 per cent. The OT/+ genotypes, whether arising directly from new mutation or indirectly from segregation, rapidly stabilise at 0.78 per cent; the proportion of homozygous off-types increases steadily. At any time, the selection of normal, +/+, genotypes for seeding will return the situation to generation one.

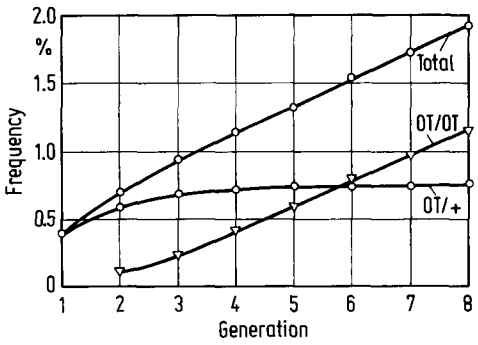

Fig. 10. Theoretical frequency of off-types. 0.4 % mutation rate. No selection

Breeders' seed taken from selected true to type plants give rise to a crop with 0.4 per cent heterozygous off-types. If basic seed were produced from this without roguing, the total frequency of off-types in the next generation would be 0.7 per cent including 0.1 per cent unmarketable rosetted plants. The basic seed tested had 0.68 per cent off-types (Table 2), which is very close to the expected 0.7 per cent. A further multiplication would give a theoretical 0.95 per cent off-types including 0.25 per cent rosetted homozygotes. Levels such as these are reasonably acceptable even for glasshouse crops in which the investment per plant is relatively high. It is however better to rogue the basic seed crop, of necessity grown in relatively short days and uniform environmental conditions, thereby ensuring that commercial seed will contain only 0.7 per cent off-types. This is now routine practice for Valentine and Dandie. It is essential that the seed production sequence, breeders' - basic - commercial, is maintained. If further generations are produced from commercial seed, unacceptable proportions of off-types will result.

Variety listing and Plant Breeders' Rights

No problems have been encountered in connection with the regulations governing the uniformity and stability requirements for variety listing and plant breeders' rights with the cultivars Valentine and Dandie. Off-type production has been accepted as a varietal characteristic just as, for example, black seed or anthocyanin pigmentation are. The variety Iceberg, an off-type producer, is over 200 years old; it has maintained its varietal status and identity over this long period of time.

Discussion

This paper deals mainly with the manifestations of off-type production at its secondary level - the production of off-types in cultivars which are unstable. This aspect is now well understood and, from a practical point of view, controllable.

The more fundamental character which determines whether a particular variety is itself stable or mutable for off-type production is more difficult to study.

Pearson (1968) discusses the mechanisms of genetic instability of rogue and off-type plants in a number of species but basic information is largely lacking. Mutability has originated at least three times in lettuce and these occurrences appear to be independent. Once established, mutability is highly heritable although it is not always present in the progeny (cf. Avondefiance). A study of the inheritance of the underlying control mechanism would be useful. The problems outlined in the section on breeding programmes would have to be faced and large numbers of plants would be involved. One approach is to hybridise known off-type producing and stable parents, utilise the Single Seed Descent method to obtain relatively uniform lines from random F_2 selections and test a large number of these for off-type production. This is being attempted.

Whilst it is useful to describe the mutation which gives rise to off-types in unstable cultivars as being the incompletely dominant condition, this is just a convenience. The direction of mutation is by no means certain. The variety type could represent the dominant condition with the change being towards recessivity. On the other hand, true breeding off-types with their flattened, rosetted habit are more primitive and resemble wild lettuce species. In this sense the mutation may represent a reversion towards wild type which is arguably the dominant condition.

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