THE IMPACT OF PLANT BREEDING ON SEED OIL CONTENT AND QUALITY IN EVENING PRIMROSE CROPS

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ABSTRACT

In recent decades, evening primrose (*Oenothera* spp.) has been grown as an agricultural crop in several parts of the world including western and central Europe, North America and New Zealand. Its value is derived from its seed oil which contains unusually high levels of gamma linolenic acid (GLA), an uncommon fatty acid which is used in pharmaceuticals and nutritional supplements. Whilst such improvements have led to a reduction in the seed price, increases in seed oil content and quality (i.e. percentage of GLA) can lead to further reductions in the cost of the end product. The breeding programme of Scotia Pharmaceuticals Ltd, UK was effective in achieving such increases. For example, in spring-sown crops in New Zealand, seed oil content increased from around 25% in older cultivars grown in the period 1990-1992 to around 28% in the cultivar "Rigel" grown in the period 1992-1999. Furthermore, GLA content increased by almost 50%, from around 9.5% in older cultivars to more than 13% in "Rigel". It has been estimated that these improvements through plant breeding have led to an 18% reduction in product costs.

Keywords: Evening primrose; *Oenothera* spp; breeding; oil content; oil quality

INTRODUCTION

In recent decades, evening primrose (*Oenothera* spp.) has been grown as an agricultural crop in several parts of the world including western and central Europe, North America and New Zealand. Its value is derived from its seed oil which contains unusually high levels of gamma linolenic acid (GLA), an uncommon fatty acid which is used in pharmaceuticals and nutritional supplements (Horrobin, 1992). Although found in some other plant and fungal oils, in evening primrose oil GLA is thought to occur in its most bioactive state, as part of a triacylglycerol called Enotherol® (Horrobin, 1994). A brief botanical description of evening primrose was given by Fieldsend (1996a).

Early commercial crops of evening primrose were grown from seed stocks available through the horticultural trade (Fieldsend, 1996a). One such "traditional" stock, grown in New Zealand, was simply known as "421". This and similar lines were unsuitable for agriculture in many ways, for example in having seed capsules which split as they matured, meaning that a significant proportion of seed was lost prior to harvest. This factor simply compounded the problem that the potential seed yield of evening primrose was already much lower than that of other combinable crops (Nix, 1995).

UK-based Agricultural Holdings, owned by the Balint family, was an important early seed producer, sourcing much of its seed from Hungary. Its subsidiary, which in time became Scotia Pharmaceuticals Ltd, a leading manufacturer of products derived from evening primrose oil, set up an evening primrose breeding programme in the mid-1970s. Whilst development of cultivars with non-splitting capsules was an important breeding objective, as higher seed yields would lead to a reduction in the price per tonne of seed, Scotia recognised that of equal importance was an increase in the oil (for nutritional supplements) and GLA (for pharmaceuticals) contents of the seed. The difference is that whilst the former are normally consumed by users as a fixed volume of oil per day, for the latter the daily dose of GLA is the important criterion.

Evening primrose breeding is made difficult by its unusual genetic system (Cleland, 1972). The formation of chromosome rings at meiosis, ordered chromosome movement and the presence of lethal genes limit segregation (and therefore scope for selecting improved lines) in the generations following the initial cross. Variable establishment, coupled with seed dormancy in many genotypes, and the long growing season of the overwintered crop in temperate climates (approximately 14 months) also present practical problems (Fieldsend and Morison, 2000a). For unimproved lines, field sowing would be an unreliable method of trialling, and selection of lines to trial would need to be made before the previous generation of breeding material had been harvested.

This paper describes the methods developed by Scotia to breed evening primrose and the improvements in oil content and quality of commercial crops in New Zealand which have resulted from the use of cultivars developed by the programme.

MATERIALS AND METHODS

The Scotia breeding programme began with the collection, from many sources, of over 2000 individual "accessions" which were numbered in a single series from X1 upwards. Each accession was evaluated in a single spring-transplanted row of 21 plants. Seeds were sown in potting compost in 750 mm diameter pots in January and placed in a refrigerator set at 4ºC for 2-3 weeks to break dormancy, following which the pots were transferred to a propagator hotbox at a temperature of 27ºC. Soon after emergence 24 seedlings of each accession were pricked out into potting compost in modules and kept in a heated glasshouse with a mean temperature of 13ºC. The rosettes were moved out of the glasshouse to acclimate during late April and transplanted into 5 m rows with a 0.55 m row spacing in the field in early May. Assessments on each row were carried out during the growing season and at maturity representative single plants were hand harvested and dried using a forced-air drier before the seed was removed from the capsules by hand-threshing. Following cleaning, seed samples were analysed for oil and GLA content using methods described by Fieldsend and Morison (2000b).

Eventually, a crossing programme was established to generate new breeding lines. Crosses were carried out using plants grown in pots in an insect-proof glasshouse. Prior to the pollen becoming viable, the anthers were removed from flowers of the plants to be used as female parents and, 24 hours later, the stigmas were hand pollinated using anthers from the male parent. Reciprocal crosses were carried out as the progeny of reciprocal crosses within *Oenothera* can be genetically completely different from each other (Cleland, 1972). The seed was hand-harvested and stored during the winter.

Subsequent generations were evaluated in breeding rows as described above. In extreme cases, the F_1 population of *Oenothera* is genetically uniform and contains one complete genome from each parent. No genetic segregation takes place in subsequent generations. Even in less extreme cases, only limited segregation occurs. Hence, Scotia adopted a modified pedigree breeding method, entailing a large number of crosses and very small (21 plant) F_2 populations. Unpromising breeding lines could be discarded after the F_2 generation whilst in some cases populations were uniform and showed enough commercial promise for seed multiplication to start.

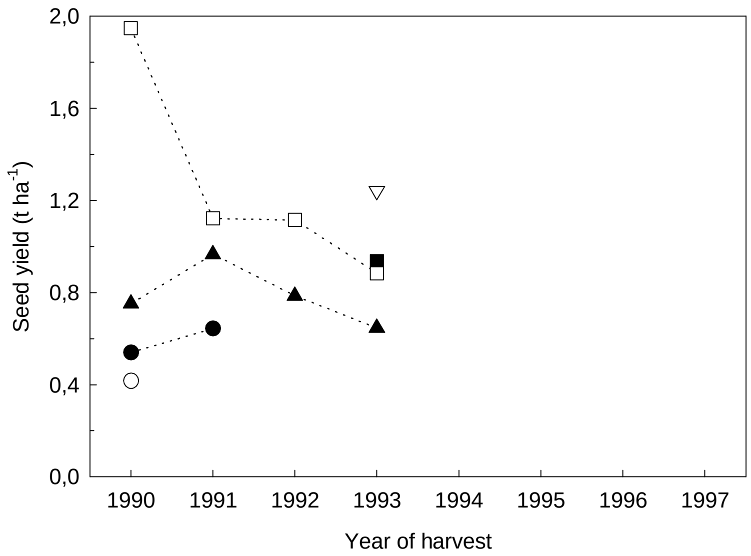
Clearly, this method does not allow the breeder to evaluate seed yields or the ability of the breeding line to overwinter. During the 1980s, overwintering ability was assessed on autumn-transplanted rows of the breeding lines showing the most promise in F_3 spring-sown rows. Selection of lines for trialling was based on field assessments from the F_1, F_2 and (until mid-August when the choice of lines for trialling had to be made) F_3 breeding rows plus the oil and GLA content data from the F_2 rows. By the 1990s, however, the establishment ability of the breeding lines had improved to such an extent that the most commercially promising advanced breeding material could be evaluated in autumn and spring-sown replicated yield trials (c.f. Fieldsend and Morison, 2000a).

New Zealand is an important source of evening primrose seed, not least because the six-month difference in harvest date compared to the northern hemisphere spreads the demands of harvest, storage and payment for seed more equably throughout the year. Overwintered crops are normally sown in February and harvested in March of the following year, whilst spring-sown crops are sown in September or October and harvested in April. The husbandry of spring-sown crops is described by Fieldsend (2003). The total area of crops grown under contract to Scotia in 1990-1997 ranged between 200 ha and 450 ha, with an approximately equal split between overwintered and spring-sown crops in the earlier years and a subsequent emphasis on spring production. This area would be divided between over 20 farmers, who would normally use field sizes of between 4 and 16 ha (Fieldsend, 2003). After harvest, a seed sample from each field was analysed for oil and GLA contents and the annual means of these data (not corrected for differences in field size) are used in this paper.

RESULTS AND DISCUSSION

Oenothera is a very diverse genus and most accessions were immediately rejected as being unsuitable for commercial seed production after one year of trials. However, three accessions were "off-type" single plants with non-splitting capsules taken from commercial crops of the "traditional" stock growing in the UK and Hungary. X444 and X851 were commercialised as the cultivars "Paul" and "Peter" respectively (similar accession X852 was not commercialised) and were awarded Plant Variety Rights (PVR) in several countries including the UK and New Zealand. The reduced risk of seed loss from "Paul" and "Peter" resulted in an approximately 50% increase in seed yields over "421" when grown as overwintered crops in New Zealand in 1990 and 1991 (Fig. 1), but these cultivars were similar to "421" in every other respect. Seed oil contents were in the region of 26.5% from autumn sowing (Fig. 2) and 25% from spring sowing (Fig. 3) whilst the GLA contents of the oil were around 9% and 9.5% respectively. Higher oil contents and lower GLA contents from overwintered crops are typical of evening primrose (Fieldsend, 2000b).

No other accessions were directly commercialised but many were used in the crossing programme. For example, the cultivar "Merlin" resulted from the cross (X444 x X390). This was a high yielding, easy to grow cultivar much liked by farmers which was also awarded PVR in several countries. The very high yield in 1990 (Fig. 1) was obtained from just 2.6 ha so this result must be treated with caution but does show its yield potential. In 1991-1993 “Merlin” outyielded "Paul” and “Peter” whilst its oil and GLA contents tended to be higher as well (Figs 2 and 3).

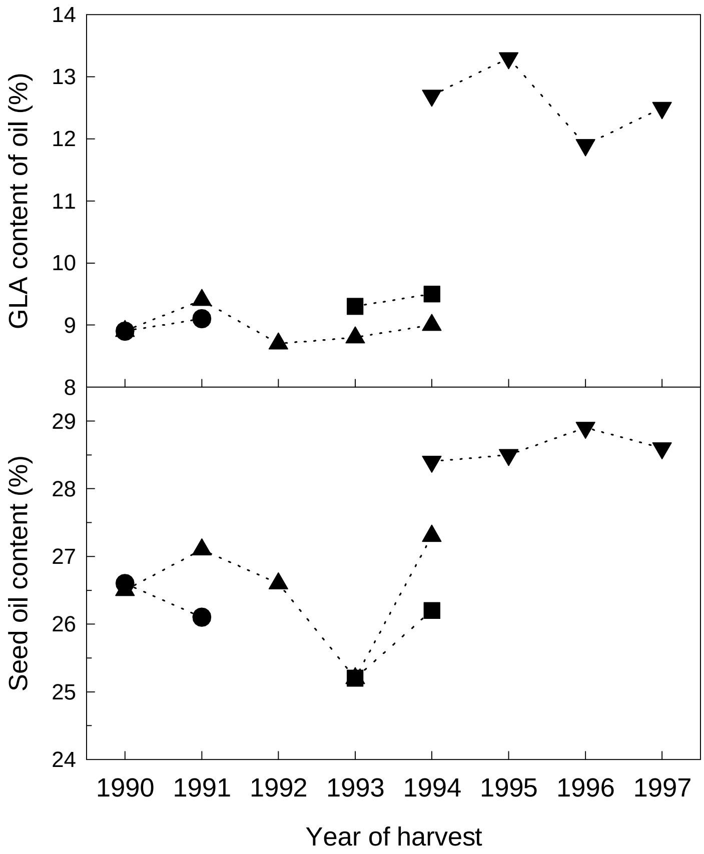


<u>Figure 1</u>: Mean seed yield of crops of evening primrose harvested in New Zealand from 1990 to 1993. Closed symbols: overwintered; open symbols: spring sown; circles: 421; triangles up: Paul/Peter; squares: Merlin; triangles down: Rigel.

A series of plants collected from the wild in Ontario, Canada produced seed with high oil contents and a very high GLA content in the oil (approximately 15%) but were unsuitable for commercial use. One of these, X945, was crossed with another accession, X1021, in 1985 and the resulting breeding line (LQB) also produced high quality oil but the seed yield was low. In turn, in 1988, LQB was crossed with “Merlin” and the result was the cultivar “Rigel”. This cultivar successfully combined the non-splitting capsules of “Paul” with the high oil and GLA contents of X945 and a high seed yielding ability.

The great potential of the breeding line was confirmed by the laboratory results from the F_3 breeding row and the unusual step was taken of sending seed from the breeding row (despite this not having been raised in isolation from other lines) to New Zealand for trialling. A 1000 m^2 plot was sown on 21 October 1991 and yielded 1121 kg seed ha^{-1} when harvested in 1992. The oil and GLA contents were 28.5% and 13.3% respectively, substantially higher than cultivars then in commercial production (Fig. 3).

"Rigel" seed multiplication commenced with a 1000 plant, 200 m^2 spring transplanted plot grown in the UK in 1992 which yielded 25 kg seed. 14.5 ha of "Rigel" crops were sown in New Zealand in October 1992 and harvested oil and GLA contents were again much higher than "Merlin". Unfortunately, yield data after 1990 are not available but Fieldsend (2003) reported yields of up to 1.87 t ha^{-1} from spring-sown "Rigel" in New Zealand in 1998 whilst in 1999 some "Rigel" crops yielded more than 2 t ha^{-1} (Scotia, commercial data). This cultivar was again awarded PVR and was the sole cultivar to be grown commercially for Scotia after 1994.



<u>Figure 2</u>: Seed oil content and GLA content of oil of overwintered crops of evening primrose harvested in New Zealand from 1990 to 1997. Cultivars are designated thus: circles: 421; triangles up: Paul/Peter; squares: Merlin; triangles down: Rigel.

Thus, by the late 1990s, oil and GLA contents of overwintered crops in New Zealand averaged around 28.5% and 12.5% respectively whilst the equivalent data from spring-sown crops were 28% and 13.5%. The impact on the GLA content of the seed was an increase from around 2.5% in 1990 to over 3.5% by 1999, with spring-sown crops normally giving the highest results (Fig. 4). The big difference between sowing dates in 1994 is due to the fact that overwintered crops were almost exclusively “Paul” and “Merlin” whilst all spring-sown crops were “Rigel”. The Scotia breeding programme closed in 1999 but “Rigel” continues to be commercially grown.

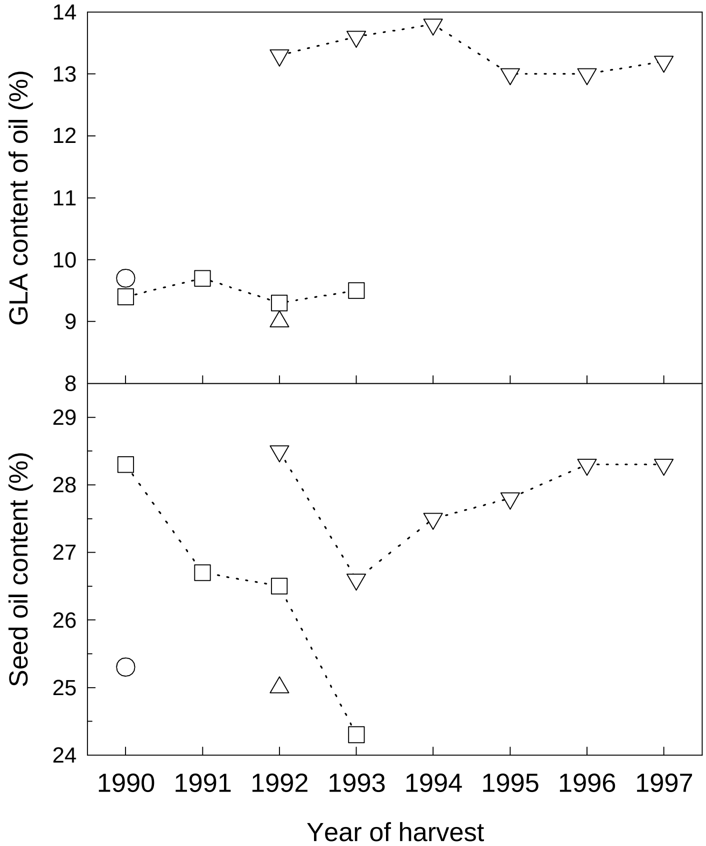
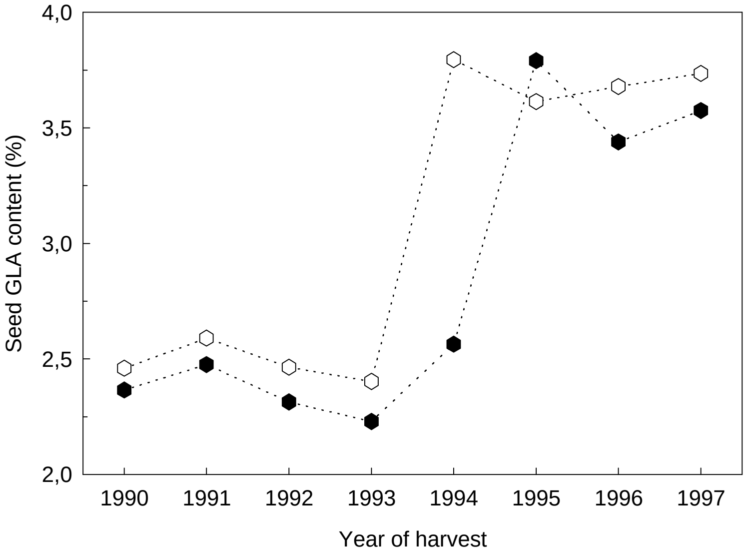


***Figure 3**: Seed oil content and GLA content of oil of spring sown crops of evening primrose harvested in New Zealand in from 1990 to 1997. Cultivars are designated thus: circles: 421; triangles up: Paul/Peter; squares: Merlin; triangles down: Rigel.*

The benefits of the cultivars bred by Scotia can be seen in different ways. For nutritional supplements, the daily dose is normally specified as the number of (gelatine) capsules to be taken but a higher GLA content in the oil gives the retailer a marketing benefit. For pharmaceuticals, where the dose normally much higher and is calculated as daily GLA intake, the number of capsules taken can be reduced. This has the twin benefits of improving patient compliance and also, owing to savings along the entire manufacturing chain, reducing product costs. It has been estimated (Scotia, commercial data) that the change to "Rigel" oil led to an 18% reduction in costs.



<u>Figure 4</u>: Mean seed GLA content of crops of evening primrose harvested in New Zealand from 1990 to 1997. Closed symbols: overwintered; open symbols: spring sown.

The oil and GLA contents achieved with "Rigel" are close to the upper limit observed in the genetic material evaluated by Scotia. However, the high linoleic acid content of the seed oil (approximately 75% in "Merlin" (Fieldsend and Morison, 2000b)) is a substrate for potentially much higher GLA contents which could possibly be achieved through genetic engineering. Borage (*Borago officinalis* L.) oil, for example, is more complex and contains approximately 37% linoleic acid and 21% GLA, i.e. a ratio of less than 2:1 (Senanayake and Shahidi, 2000). de Gyves (2004) demonstrated the concept by using an *Agrobacterium*-mediated transformation procedure to deliver a cDNA encoding a 6-desaturase from borage under the control of a cauliflower mosaic virus (CaMV) 35S promoter in “Rigel”. Analysis of the transformed plants demonstrated an altered profile of polyunsaturated fatty acids, with an increase in GLA and octadecatetraenoic acid in leaf tissues when compared with control lines. The fatty acid profile of the seed oil was virtually unaltered, but this is not surprising as CaMV 35S is not a seed-specific promoter.

Fieldsend (1996b) coined the term “factories in the field” to describe the cultivation of evening primrose as a source of GLA. The farmer is the “factory manager”. Agricultural research provides him with the correct “equipment” in the form of cultivars adapted to his growing conditions, and the know-how, or “operating manual”, in the form of husbandry guidance. Almost ten years later, the concept has been adopted as part of the stakeholder proposal for the “Plants for the Future” technology platform (Anon, 2005). In evening primrose, the advances envisaged by the technology programme have already been achieved in practice.

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