# DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS

**CSG 15**

Research and Development

# Final Project Report

(Not to be used for LINK projects)

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## Executive summary (maximum 2 sides A4)


The seed oil of Pot marigold *Calendula officinalis* contains calendic acid, which is of interest within the paint industry as a sustainable alternative to Tung oil. The agronomic performance of *C. officinalis* is not optimised and current knowledge of the physiology is minimal. Meanwhile, breeding efforts to-date have been towards improving the floristic value of *Calendula* rather than the agronomic suitability of the species. In order to improve this a European Commission programme of evaluation of the crop (FAIR-PL98-3713) was initiated in 1998. The two UK components of the work, conducted by ADAS and funded by Defra, were as follows.

1. This project sought to identify key crop performance factors through improved physiological understanding of *C. officinalis* as an oil producing seed crop, by using nitrogen and population density to influence canopy growth and photosynthetic capacity, biomass yield, flower number and oil yield, with the aim of using crop physiological studies to develop crop management systems for southern England and to identify genotype x environment interactions for crop performance.
2. The UK hosted one of a number of genotype x environment interacton sites in order to evaluate newly developed lines, and to identify genotype x environment interactions for crop performance.

Assessments included soil nutritional status, crop development dates, light interception, above-ground biomass accumulation, seed yields, seed oil content and oil quality.

Optimum plant density for seed yield was less than 40 plants $m^{-2}$. At lower plant densities, individual plants were larger, but did not produce comparable dry matter yields per unit ground area as higher plant density treatments. Increasing plant density increased peak photosynthetic area index (PAI); the largest value achieved was 4.59 by the 80 plants $m^{-2}$ treatment. *C. officinalis* is indeterminate and yield is relatively stable over a wide range of plant population densities (for example, final plant densities of 60 - 100 plants $m^{-2}$ in 2000 with cultivar 981188-4).

Optimum crop N requirement for biological and economic yield was 100 kg N $ha^{-1}$, a similar N requirement to other spring-sown oilseed crops in the UK such as oilseed rape. Nitrogen applications up to 150 kg N $ha^{-1}$ increased peak photosynthetic area index (PAI), but in the absence of applied nitrogen, PAI peaked at 1.95. Despite the rapid rate of PAI increase during early crop development, a large proportion of the potential solar radiation cannot be captured because the crop is established in May.

Flower number $m^{-2}$ increased with plant density, but lack of seed yield response to increasing density suggested that flower number did not limit yield expression.

Interactions between genotype and environment were observed by analysis of data from successive annual experiments in southern UK and from five sites at different locations within the EU in 2000. Newly developed lines, generally, outperformed existing varieties indicating that the breeding programme was developing new material of enhanced agronomic potential. Improved lines showed good adaptation to differing environments, with the exception of the most southern site (Bazierge, France). Crops were grown at this site in 1999 and 2000 and performed poorly in both seasons.

Horticulturally-grown *C. officinalis* has a biennial lifespan, and this could offer advantages of input cost reduction in agriculture. Additionally, biennial crops could perform better in the second year, since they would be better able to exploit early season radiation. An experiment to study the performance of *C. officinalis* as a biennial crop was carried out in 2000 and 2001. Winter survival was assessed after seed harvest in late summer 2000. The crop did not survive the winter and it was concluded that *C. officinalis* is unlikely to succeed as a biennial crop in the UK, unless more winter-hardy varieties are developed.

Alkyd paints with less volatile organic compounds (VOC) and non-fogging polyurethane foams (PUF) can be produced with calendula oil as raw material. However, with current calendula oil yield and processing efficiency, the cost price of the oil is too high for competition with petrochemical raw materials for high emission paints and PUF. Further work could be aimed at reducing the cost price by increasing oil yield through *Calendula* breeding and agronomy.

It is suggested that breeding priorities should be directed towards winter hardiness, to allow earlier sowing and greater light interception, uniformity of maturity and better seed retention within mature seed heads.

A summary of the progress made in the EC FAIR project is as follows, adapted from the project final report (van Loo, 2002).

The main aims of this project have been reached:

1. We have developed new industrial application possibilities of calendula oil and its derivatives and extended the knowledge on existing applications: the use of calendula oil as a raw material for reactive diluents of alkyd paints and for polyols for non-fogging polyurethane foams.
2. We have optimized and developed oil recovery techniques in order to meet the specific requirements imposed by the different end users: the technically most feasible technique for oil extraction proved to be a pre-treatment using a specialized seed sorting machine (ASTER-technology), followed by hexane extraction and standard oil refinery (degumming, neutrilization and bleaching). This way a low colour and reactive oil can be produced that suits the end users.
3. We have identified a use for the seed by-product in animal feeds; the seed residue after oil extraction is protein-rich and can safely replace soy bean meal in animal diets.
4. The goal of developing *C. officinalis* into an economical feasible crop for the main production areas of Europe has not been fully reached. Despite a doubling of the oil yield of *Calendula* (from 250 to 500 kg $ha^{-1}$ oil) by plant breeding and despite improved agronomical practices, at the yield level of the improved genotypes, still the cost price of *Calendula* is too high to safely start a production chain. Yet, areas in Europe and in Mediterranean countries can be found were the cost price would be low enough. Development of extraction facilities in those areas would be economically feasible. Part of the consortium is now investigating the economic feasibility of primary production and extraction in Marocco. Based on this Maroccon calendula oil production, paint production is planned to take place in Europe. Another option is to continue plant breeding in order to breed cultivars with an oil yield of 750 kg $ha^{-1}$. With such a yield also *Calendula* primary production can take place in for example the UK or areas in Germany or in candidate member states like the Czech Republic, Hungary and other countries within the enlarged EU.
5. We have developed cost-effective crop management systems for north-west Europe: for this a growers manual is available.
6. We have improved genetic stock for desirable agronomic characteristics: we have new *Calendula* genotypes which have double the oil yield of the parental accessions from which they were derived.

A growers' guide has been produced as part of this project and a copy is attached to this report.

## Scientific report (maximum 20 sides A4)

**INTRODUCTION**

There is worldwide interest in exploiting renewable vegetable oils for industrial uses and as replacements for petrochemical-derived feedstocks. Amongst the annual crops, soyabean, rapeseed and sunflower oil are widely used in the lubricants, printing and coatings sectors. These high volume, low value base oils are complemented by other renewable speciality oils such as linseed and castor, which command higher market prices due to unique fatty acid profiles.

A wide range of unusual fatty acids is present in the seed oils of plants and amongst these is the seed oil of *Calendula officinalis L*. This crop has been cultivated for many years (Meizer zu Beerentrup & Röbbelen, 1987), its flowers being used as both a source of essential oil for medicinal treatments and as a natural dyestuff from its orange or yellow flowers. However, the seed of *Calendula* contains 18-22% oil and this contains 55 to 60% of the very reactive C18:3 fatty acid, calendic acid (Muuse, Cuperus & Derksen, 1992). The chemical structure of calendic acid, containing three reactive conjugated, ethylenic bonds and an octatrienoic acid isomer, makes it a potentially useful compound for industrial products and chemical modification. Viable market opportunities have been identified for calendula oil as an ingredient for the production of reactive diluents and oil based alkyd resins for use in high solid paints. Research has shown that the oil can be successfully used in paint formulations, substituting for Tung oil which is currently imported into the EU (Marvin, 1999).

*C. officinalis* is well adapted to temperate climatic zones in Europe and is believed to have originated in the Mediterranean area (Earle, Miklojajczak & Wolf, 1964). There has been limited agronomic research on its potential as an industrial oil crop species (Berti & Schneiter, 1993; Cromack, Freer & Smith, 1993; Röbbelen, Theobald & Pascual-Villalobos, 1994). Agronomic problems that limit the crop's development have included seed shedding, the polymorphic nature of seed types which create harvesting and seed cleaning difficulties (Breemhaar & Bouman, 1995), pest problems (Berti & Schneiter, 1993) and limited herbicide choices (Cromack & Smith, 1998). At present, seed yields are approximately 1000 to 1500 kg $ha^{-1}$, but with improved production systems and selected cultivars, it is predicted that yields could double (Marvin, 1999).

The development of novel oilseed crops requires the development of primary production, processing and industrial applications. For the agronomy of a new crop to be refined successfully, there must be a detailed understanding of its interaction with major agronomic, genetic and climatic factors. Then, cost-effective production systems can be developed and breeders can target selection to the most appropriate traits.

In order to promote the development of *Calendula* as a novel oil crop in Europe, a project was initiated in 1998 with 12 partners from 5 countries, funded under the EC FAIR Programme and led by CPRO_DLO (NL). Research activities were divided into two sections. Firstly, primary production which included the research by ADAS and secondly, processing, refinement and industrial applications. The objectives of the project were:

**1.** to develop new and to extend known industrial application possibilities of calendula oil and its derivatives;
**2.** to develop and optimise oil recovery techniques in order to meet the specific requirements imposed by the different end users;
**3.** to identify uses for the seed by-product in animal feeds;
4. to develop *Calendula officinalis* towards an economically feasible crop for the main production areas of Europe;
**5.** to develop cost-effective crop management systems for north-west Europe;
**6.** to improve genetic stocks for desirable agronomic characteristics.

The work was subdivided into the following tasks:

PRIMARY PRODUCTION SECTION

- Task 1: Plant breeding
- Task 2: Crop production
- Task 3: Post-harvest management
- Task 4: *Calendula* seed and flower-heads production

PROCESSING, REFINEMENT AND INDUSTRIAL APPLICATIONS SECTION

- Task 5: Oil recovery
- Task 6: Flower-heads valorisation
- Task 7: Feed evaluation
- Task 8: Oil refining and modification
- Task 9: Oil by-products valorisation
- Task 10: Oil based resin production and evaluation

Task 11: Reactive diluents production and evaluation
Task 12: (Non)ionic surfactants production and evaluation
Task 13: Polyurethane foam (Puf) production and evaluation
Task 14: Paint formulations based on methylcalendulate and calendula oil based resins

The ADAS contribution to this programme, delivery into Task 2, was jointly funded by MAFF, now Defra and the results are presented here. Task 2 was sub-divided as follows:

Subtask 2a: Crop physiology;
Subtask 2b: Evaluation of *Calendula* genotypes under a wide range of European environmental conditions.

ADAS was responsible for Subtask 2a. Subtask 2b was delivered in collaboration with an EU partner (CEBECO) and involved cultivar evaluation in France, Germany, Netherlands and the UK. ADAS had no control over the selection of cultivars or the experimental work in the other three EU countries.

Current knowledge of the physiology of *C. officinalis* is minimal. This project sought to identify key crop performance factors through improved physiological understanding of *C. officinalis* as an oil producing seed crop, by using nitrogen and population density to influence canopy growth and photosynthetic capacity, biomass yield, flower number and oil yield.

**RELATE TO POLICY OBJECTIVES**

The development of novel oilseed crops for specialist high value markets was an aim of UK (MAFF) and EU policy, as was the use of renewable oilseeds as substitutes for products derived from fossil fuel sources. MAFF (now Defra) funding in the project was limited to match funding of the EC award to ADAS to undertake agronomy research.

**OBJECTIVES OF THE Defra-FUNDED WORK**

1. To develop from detailed crop physiological studies cost-effective crop management systems for southern England.
2. To identify genotype x environmental interactions to aid the selection and production of the most appropriate genotypes for Southern England, using assessment techniques developed from the research outlined above.

**OBJECTIVE 1. TO DEVELOP FROM DETAILED CROP PHYSIOLOGICAL STUDIES COST-EFFECTIVE CROP MANAGEMENT SYSTEMS FOR SOUTHERN ENGLAND**

**Materials and Methods**

*Trial sites*

Experiments were conducted in 1998, 1999 and 2000 on commercial farm sites in Devon, UK. Two experimental sites were used, Credition (1998) and Starcross (1999 & 2000). Both had free draining sandy-loam soils.

*Treatments and experiment design*

In the 1998 and 1999 seasons, *C. officinalis,* cultivar Hens and Chickens (HC), was sown at four seed rates and two rates of nitrogen fertiliser were applied. Seed rates were chosen to achieve densities of 20, 40, 60 and 80 plants $m^{-2}$, assuming 80% germination and 60% field emergence. Nitrogen fertiliser was applied as ammonium nitrate at sowing, at rates of zero and 50 kg $ha^{-1}$ N.

In 2000, two separate experiments were established, investigating population density and nitrogen effects on crop growth and yield. In the crop density experiment, the target population densities were the same as in 1998 and 1999 (20, 40, 60 and 80 plants $m^{-2}$) and there was a single N application rate of 50 kg $ha^{-1}$ N. In the nitrogen experiment, N application rates were 0, 50, 100, 150 and 200 kg $ha^{-1}$ N, all plots with a target population density of 60 plants $m^{-2}$. A new, 'high yielding' cultivar, coded 981188-4 (Breeder: Plant Research International, Wageningen, the Netherlands), was used in 2000 and was very similar to cultivar HC in terms of development rate and morphology. To allow seasonal comparisons, additional plots were sown with cultivar HC, with a target population density of 60 plants $m^{-2}$ and an N application of 50 kg $ha^{-1}$ N.

In 1998 and 1999, the experiments had a factorial, randomised block design with all treatment combinations represented in each of three blocks. In 2000, the two separate experiments each had a randomised block design with three blocks. Individual plot dimensions were 12 m × 2.25 m in all seasons and were sown as 'paired plots', facilitating destructive plant sampling in one plot and final combine harvest yield assessment in the adjacent plot.

*Crop management and harvesting*

Experiments were sown on 08 May 1998, 05 May 1999 and 03 May 2000. At all sites, soils were ploughed and cultivated prior to sowing using an Øyjord seed drill. Pre-establishment soil nutrient analysis was used to determine the level of fertiliser amendment. Phosphate, potash and sulphur were supplied in excess of predicted requirement. Phosphate and potash fertilisers were applied to the soil prior to drilling at a rate of 60 kg $P_2O_5$ and $K_2O$ $ha^{-1}$ in all seasons. Routine herbicide treatments were applied based on the recommendations of Cromack, Smith & Morton (1997). These were only partially successful, so plots were kept weed-free by occasional hand weeding. No fungicides or insecticides were applied to experiments. All crops were desiccated with diquat (as Reglone, Zeneca; 200 g a.i. $l^{-1}$) at 600 g a.i. $ha^{-1}$, 10 to 16 days prior to final harvest with a plot combine harvester.

*Soil and plant measurements, 1998 and 1999*

A soil sample comprising of 25 soil cores to a depth of 15 cm was taken from each site in early spring and analysed for pH, P, K, Mg and organic matter content. Soil mineral nitrogen was measured in late March or early April at the end of winter drainage, by sampling to a depth of 60 cm in two depth increments, 0 to 30 cm and 30 to 60 cm. Samples were analysed for $NH_4$-N and $NO_3$-N and soil mineral nitrogen was calculated assuming standard bulk densities.

Crop emergence date, emerged plant population and date of flowering were assessed on all plots. Flowering date was defined as visible flowers on 50% of plants within the plot. Crop height was recorded at full flowering, defined as 95% of plants with flowers.

Crop light interception was monitored weekly from approximately 3 weeks after emergence until canopy closure in 1998, using a Sun Scan meter (Delta-T Devices, Hoddesdon, Hertfordshire, UK). Destructive sampling of plants to determine the distribution of dry matter into plant components was done on three occasions. These were classified as vegetative, flowering and final crop maturity. On each occasion, all the plant material was cut at ground level from within a 1 m² quadrat. Sample weight was recorded and two sub-samples of 3-5 plants were selected at random from the bulked material. Both sub-samples were weighed, divided into leaf, stem, flowers or seed fractions as appropriate. On one sub-sample, fresh and dry weights of each fraction were recorded (after drying for 24 h at 102 ºC in a forced-air oven). Material from the second sample was processed using a planimeter (LI-COR LI-3100 Area Meter, Lincoln, Nebraska, USA) to determine leaf and stem green areas.

Seed yields were determined from both the hand-harvested quadrats taken at full crop maturity (immediately prior to crop desiccation) and from adjacent plots harvested using a Sampo Rosenlew plot combine harvester. Seed yields were corrected to 9% moisture content (91% dry matter).

*Soil and plant measurements, 2000 season*

Soil pH, P, K and Mg content, organic matter content and soil mineral nitrogen were measured using the same procedures as in 1998 and 1999. In the density experiment, assessments for crop emergence and plant population were made on five occasions during the growing season. Destructive sampling procedures were amended from those adopted in 1998/99 to include measurements of plant population based on the number of main stems counted after each sampling occasion. Additionally at the flowering stage, 3-5 whole plants were cut into thirds based on total crop height. Flower number was then counted for each canopy layer. In the nitrogen experiment, light interception was measured on four occasions during the vegetative stage of growth. Crop destructive sampling was intensified to cover the vegetative phase of plant growth, with weekly sampling from emergence until flowering. Emerged plant population and crop height were also measured. Seed yield was measured in both experiments using a plot combine harvester. Seed from the nitrogen experiment was analysed for its nitrogen content using the Dumas method (Anon, 1989) and for oil content, a modified method based on Metcalf & Schmitz (1992).

*Statistical analysis*

The effects of treatments on plant growth, biomass production and final seed yield were investigated using Analysis of Variance, Genstat 5. Yield data from the nitrogen experiment in 2000 were used to estimate the economic optimum rate of applied N (assuming a seed:nitrogen price ratio of 3:1), using a linear plus exponential function (LEXP) of the form: Yield = a + b $\times$ (0.98)$^N$ + c $\times$ N where N is the rate of fertiliser N applied, a, b and c are constants (George, 1984).

**Results**

*Emergence and establishment*

Dates of developmental stages are presented in Table 1. During 1998, some small differences were observed in the time from emergence to developmental stages, but there was no clear correlation to applied treatments. During 1999, some variation was observed in time to 50% plant maturity, but dates of all other development stages were common to all treatments. The time from emergence to maturity ranged from 89 to 95 days. Shortly after reaching maturity, the crop was desiccated, prior to harvest. Total crop duration (emergence to harvest) ranged from 114 to 123 days. During 2000, cultivar 981188-4 was studied concurrently to cultivar HC and both cultivars had total crop durations of 123 days.

**Table 1.** *Ranges of dates of emergence and development stages, and crop duration values for crops of* C. officinalis *cultivar Hens and Chickens, 1998 - 2000.*

| Developmental stage | 1998 | 1999 | 2000 |
|---|---|---|---|
| Emergence date | 21-23 May | 16 May | 13 May |
| 50% Flowering | 08 - 10 Jul | 04 Jul | 06 Jul |
| 50% Mature | 24 Aug - 01 Sep | 13 - 17 Aug | 14 - 18 Aug |
| Crop dessicated | 05 Sep | 28 Aug | 01 Sep |
| Crop Harvested | 21 Sep | 07 Sep | 12 Sep |
| Duration from emergence to maturity (days) | 95 | 89 | 93 |
| Duration from emergence to harvest (days) | 123 | 114 | 123 |

Main stem population densities during 2000 for cultivar 981188-4 are shown in Fig. 1. At the lowest density (20 plants m$^{-2}$), stem populations remained relatively constant indicating the absence of competitive effects. At all higher planting densities, populations showed an initial increase followed by a decline in numbers later in the season. Final plant populations were significantly higher than the target populations.

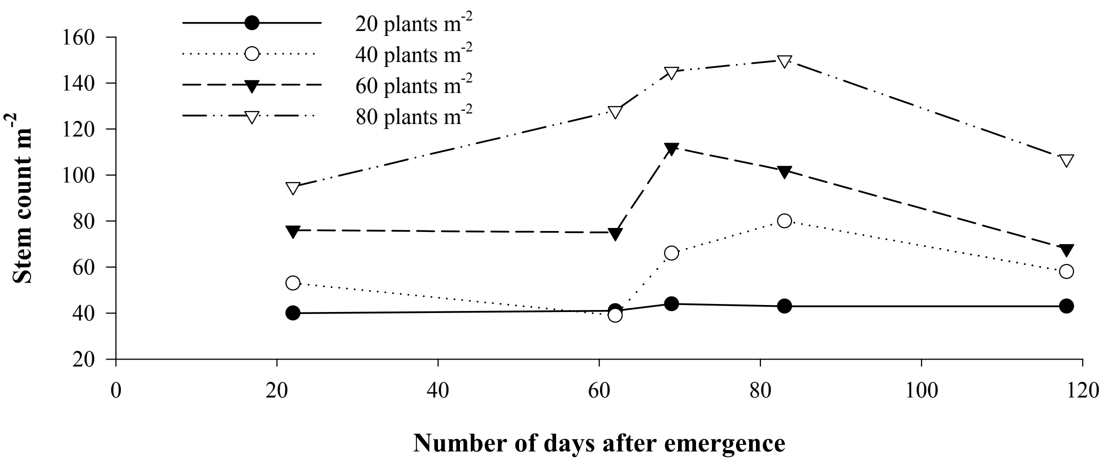



**Fig. 1.** *Main stem populations (equivalent to plants m$^{-2}$) for treatments with different seed rates, identified in the key as target plant populations (plants m$^{-2}$), cultivar 981188-4, 2000.*

*Yield response*

Results with cultivar HC indicated that ex-combine seed yields were unresponsive to plant density and nitrogen treatments (Table 2). In 1998, there was no significant increase in seed yield between 20 to 80 plants m$^{-2}$ and yields decreased (P=0.051) when 50 kg N ha$^{-1}$ was applied. In 1999, nitrogen had no effect on yield, but yield was significantly less (P=0.013) at 20 plants m$^{-2}$, than from 40 to 80 plants m$^{-2}$ treatments, where yields were similar. Ex-combine seed yields over the two years of study with cultivar HC ranged from 0.65 to 1.14 t ha$^{-1}$. Results from 2000 with cultivar 981188-4 indicated a similar trend with regard to planting density (Table 3): no significant response was found between planting density treatments with yields ranging from 2.0

to 2.2 t $ha^{-1}$. Yields were higher for cultivar 981188-4 than for cultivar HC which yielded 1.52 t $ha^{-1}$ in 2000 at 60 plants $m^{-2}$.

**Table 2.** *Effect of Density and Nitrogen treatments on seed yield (t $ha^{-1}$ at 91% DM) in cultivar Hens and Chickens, 1998 and 1999 seasons. SED = standard error of mean, 17 d.f.*

| | 1998 | | | 1999 | | |
|---|---|---|---|---|---|---|
| Density (plants $m^{-2}$) | N0 | N50 | Mean | N0 | N50 | Mean |
| | SED=0.099 | | SED=0.069 | SED=0.071 | | SED=0.050 |
| 20 | 1.08 | 1.04 | 1.06 | 0.68 | 0.65 | 0.66 |
| 40 | 1.08 | 1.02 | 1.05 | 0.80 | 0.82 | 0.81 |
| 60 | 1.14 | 0.94 | 1.04 | 0.79 | 0.92 | 0.86 |
| 80 | 1.08 | 0.96 | 1.02 | 0.81 | 0.74 | 0.78 |
| | SED=0.049 | | | SED=0.036 | | |
| Mean | 1.10 | 0.99 | | 0.77 | 0.79 | |

**Table 3.** *Effect of Density on seed yield (t $ha^{-1}$ at 91% DM) in cultivar 981188-4 and cultivar Hens and Chickens at one planting density, 2000. SED = standard error of mean, 6 d.f.*

| | Cultivar | |
|---|---|---|
| Density (plants $m^{-2}$) | 981188-4 | Hens and Chickens |
| | SED=0.182 | |
| 20 | 1.99 | - |
| 40 | 2.29 | - |
| 60 | 2.13 | 1.52 |
| 80 | 2.20 | - |
| Mean | 2.16 | - |

Using a LEXP curve-fitting model, the optimum economic rate of nitrogen fertiliser for cultivar 981188-4 in 2000 was 86 kg $ha^{-1}$, with an estimated yield of 2.33 t $ha^{-1}$ (Fig. 2). Yields ranged from 1.73 t $ha^{-1}$ (0 kg $ha^{-1}$ N) to 2.56 t $ha^{-1}$ (200 kg $ha^{-1}$ N), an increase of 0.83 t $ha^{-1}$ (48%). In 2000, only 14 kg $ha^{-1}$ N was measured in the 0-60 cm soil layer, which together with the derived optimum fertiliser N rate of 86 kg $ha^{-1}$ N suggested a crop N requirement of 100 kg $ha^{-1}$ N. This response to N in 2000 contrasts with results in 1998 and 1999 (see above), when amounts of residual nitrogen were high, at 133 and 117 kg $ha^{-1}$ N respectively.

*Seed oil yield and N content (2000)*

Seed N content in cultivar 981188-4 increased significantly (P<0.001) with increasing rate of fertiliser N up to the maximum rate applied of 200 kg $ha^{-1}$ N (Fig. 3). Up to 150 kg N $ha^{-1}$, seed N increased by 0.18% for each increment of 50 kg $ha^{-1}$ N. In contrast, there was no significant effect in oil content from changes in the rate of fertiliser N applied (Fig. 3). In cultivar HC, which received 50 kg $ha^{-1}$ N, seed N and oil contents were 2.11 and 1.75% respectively, only marginally less than in cultivar 981188-4 at the same rate of fertiliser N. The N content in plant dry matter during the vegetative phase increased with increasing rate of fertiliser N, from 3.59% to 5.47% at 33 days after emergence (DAE) for 0 and 200 kg N $ha^{-1}$ treatments respectively. Plant N content fell as growth proceeded, but exceeded 100 kg $ha^{-1}$ N in above ground dry matter by 53 DAE. Nitrogen recovered in seed ranged from 32 to 60 kg $ha^{-1}$ N and the oil yield $ha^{-1}$, an important economic indicator for industrial oil crops, ranged from 259 kg $ha^{-1}$ oil at 0 kg $ha^{-1}$ N up to 400 kg $ha^{-1}$ oil at 200 kg $ha^{-1}$ N.

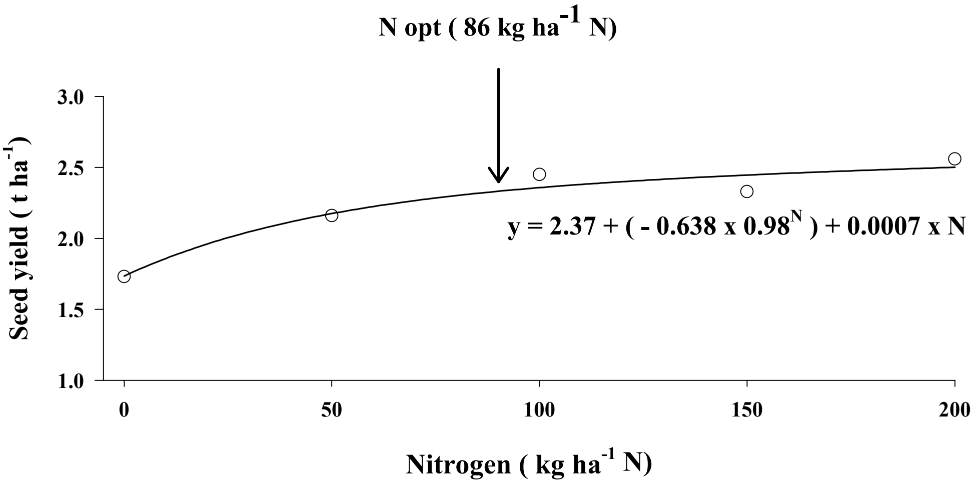



**Fig. 2.** *Yield response of* Calendula officinalis *to nitrogen fertilisation at a target plant density of 60 plants* $m^{-2}$*, 2000.*

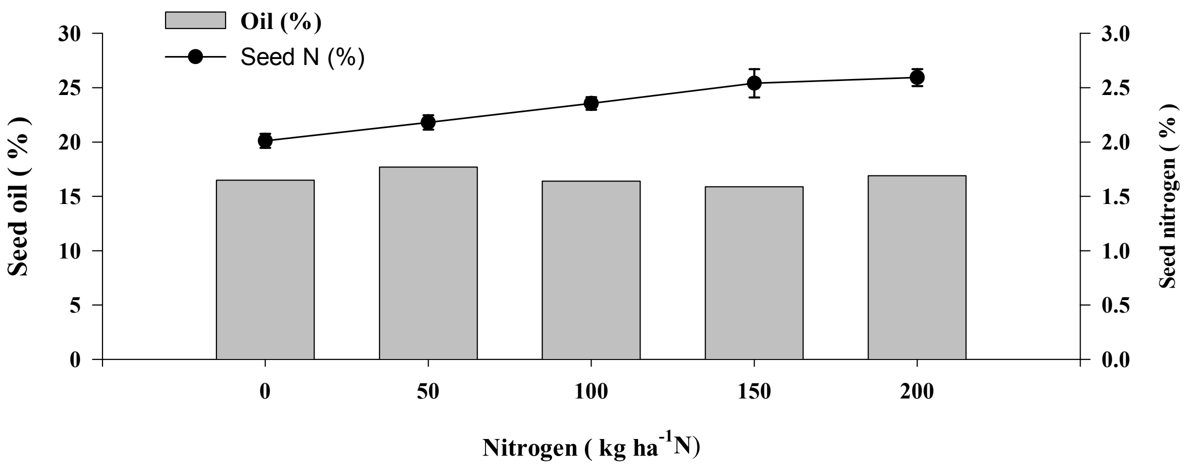
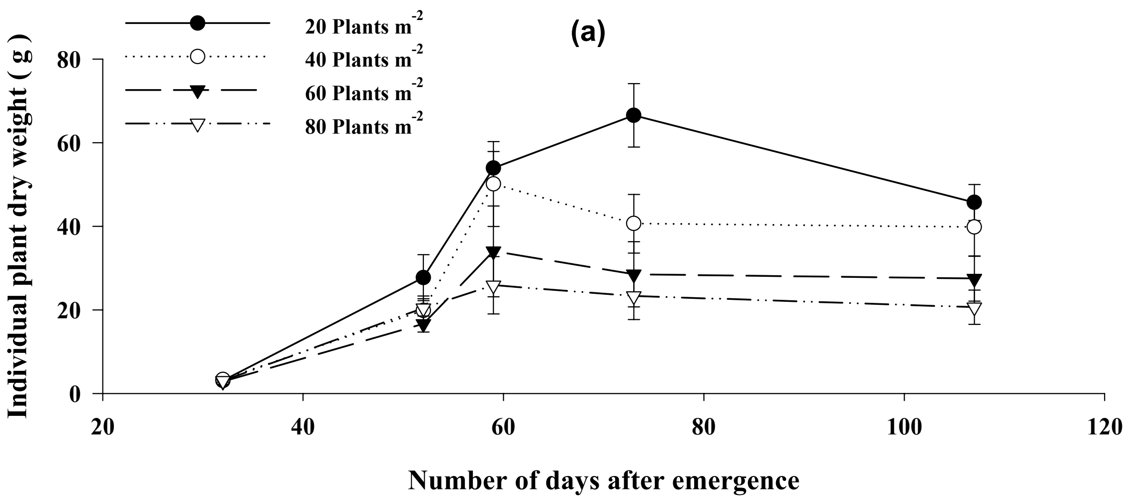



**Fig. 3.** *Changes in seed oil and nitrogen content (%) at different rates of nitrogen fertilisation.*



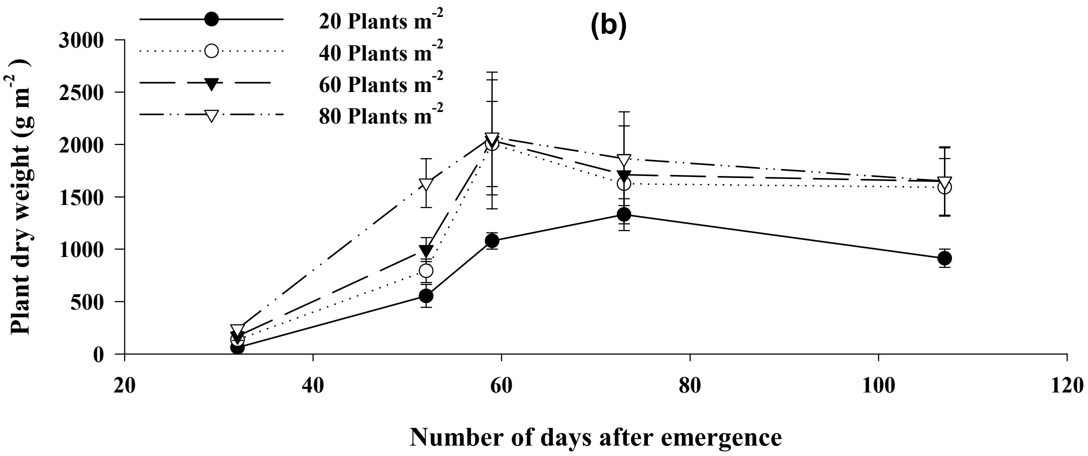



**Fig. 4.** *Above ground dry weight for* C. officinalis *at 4 different planting densities expressed (a) per plant and (b) per* $m^{-2}$*, 2000.*

*Biomass accumulation and partitioning*

Biomass accumulation per plant (Fig. 4a) in cultivar 981188-4 showed a yield building phase up to 76 DAE for the lowest population density (20 plants $m^{-2}$) but a shorter duration (61 DAE) for higher densities. After the yield building phase, dry weight per plant decreased, presumably due to foliar senescence and/or resource allocation to high energy compounds (oils rather than carbohydrate).

In 2000, when dry matter was expressed per unit ground area (g $m^{-2}$) the lowest planting density (20 plants $m^{-2}$) had the lowest final dry matter yield ($P<0.05$) (Fig. 4b). There was no significant difference between dry matter yields of the other planting densities.

In response to increasing planting density, plants demonstrated phenotypic plasticity, with individual plant size decreasing with density (Fig. 4a). The convergence of yields per unit ground area at the 40, 60 and 80 plants $m^{-2}$ treatments (Fig. 4b) is explained by this yield compensation, and also by the loss of stems or whole plants (Fig. 1). Plants at the lowest planting density (20 plants $m^{-2}$), although larger, did not produce similar dry matter yields per unit ground area as higher planting density treatments.

The seasonal allocation of biomass to different plant organs in cultivar HC (2000) is presented as an example of biomass distribution dynamics (Fig. 5b) with the total above ground dry matter production given in Fig. 5a. During the yield building phase, the proportion of stem dry matter increased up to a maximum of 66% of total plant dry weight, with a concurrent decrease in leaf dry matter component. After the yield building phase, development of reproductive organs (flowers and seed heads) continued, while leaf dry matter remained constant and stem dry matter decreased. This suggests re-allocation of resources from stems to seed. At the final destructive harvest (106 DAE) before combine harvesting, the proportion of dry matter in flowering organs increased to a maximum of 34%.

Profiles of biomass allocation throughout the canopy for cultivar 981188-4 (Fig. 6), demonstrate the dominance of the stem component before and during flower development. The dry weight contributions from the stems decreased during flower and seed development. During early development (12 July), the greatest biomass component was at the base of the canopy, reflecting predominantly vegetative growth. During flower development, the upper section of the canopy became the dominant component of biomass until maturity.

Flower number per plant decreased with increasing plant density, whilst flower numbers $m^{-2}$ increased (Fig. 7). Data collected in this experiment were similar to those of Meizer zu Beerentrup & Röbbelen (1987), who showed that mean flower heads per plant ranged from 10.6 to 28.3 in a selection of *Calendula* accessions. This increase in flower number $m^{-2}$ was not associated with an increase in seed yield, suggesting flower number was not limiting yield.

*Canopy development and radiation interception*

In 2000, photosynthetic area index (PAI) values showed that increasing planting density (Fig. 8) resulted in greater PAI maximum values. The largest PAI value was 4.59, achieved in the 80 plants $m^{-2}$ treatment (56 DAE). PAI values are plotted against incident radiation to indicate the potential radiation interception capability of the canopy. This crop was established in mid-May (13-23 May) and a large proportion of annual solar radiation was not intercepted, despite the rapid rate of PAI development once the crop had emerged.

Radiation interception (Fig. 10) followed a similar sharp increase with early canopy development. Higher planting densities intercepted more radiation early in the season and canopies of all densities achieved 90% interception by the final assessment.

The relationship between nitrogen application and radiation interception was less clear. With the application of N (Fig. 9), the maximum PAI value achieved was 3.78, achieved 56 DAE by the 150 kg ha$^{-1}$ N treatment. Applications of N above 150 kg ha$^{-1}$ appeared to decrease peak PAI. Green leaf area duration (GLAD) values were also greater with the 150 kg ha$^{-1}$ treatment. In the absence of any applied N, maximum PAI was 1.95 (Fig. 11), radiation interception was reduced, and by the end of the season there was a trend towards greater radiation interception at the highest N treatment. Radiation interception measurements were designed to cover the period of canopy development, with the final assessments made 76 DAE (Fig. 10). However, patterns of PAI indicate a rapid loss of leaf area approximately 66 DAE, but the implications of this on radiation interception capability were not assessed.

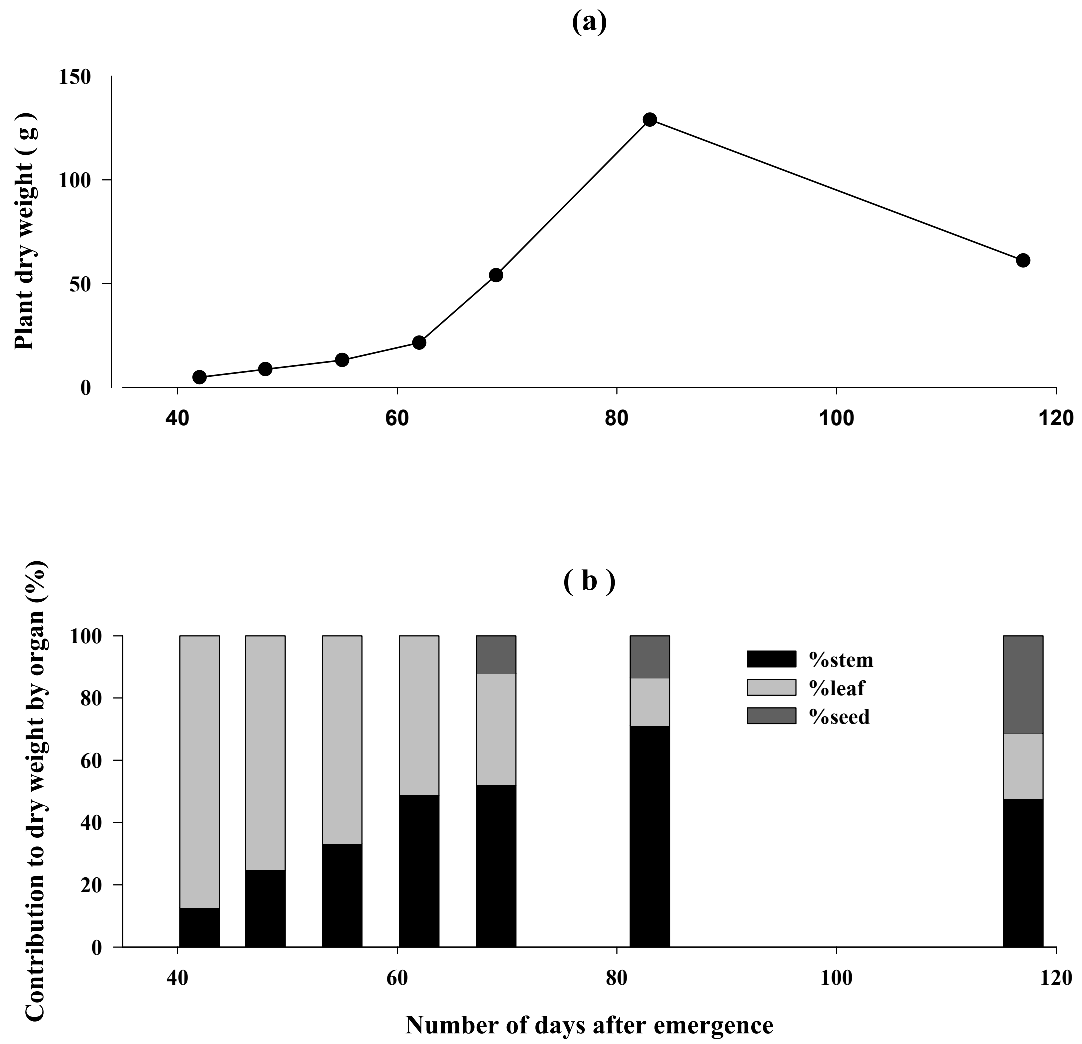



**Fig. 5.** *Pattern of above ground biomass accumulation (a) of* C. officinalis *cultivar Hens and Chickens throughout the growing season, 2000; (b) contribution to total dry weight by different organs.*

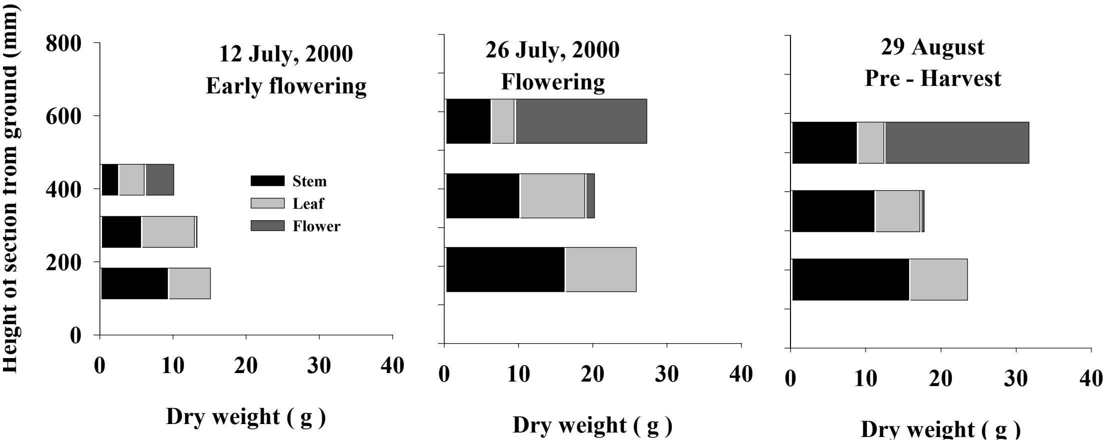



**Fig. 6.** *Breakdown of dry weight contributions (g) within three equal height strata of the* C. officinalis *canopy at three developmental stages.*

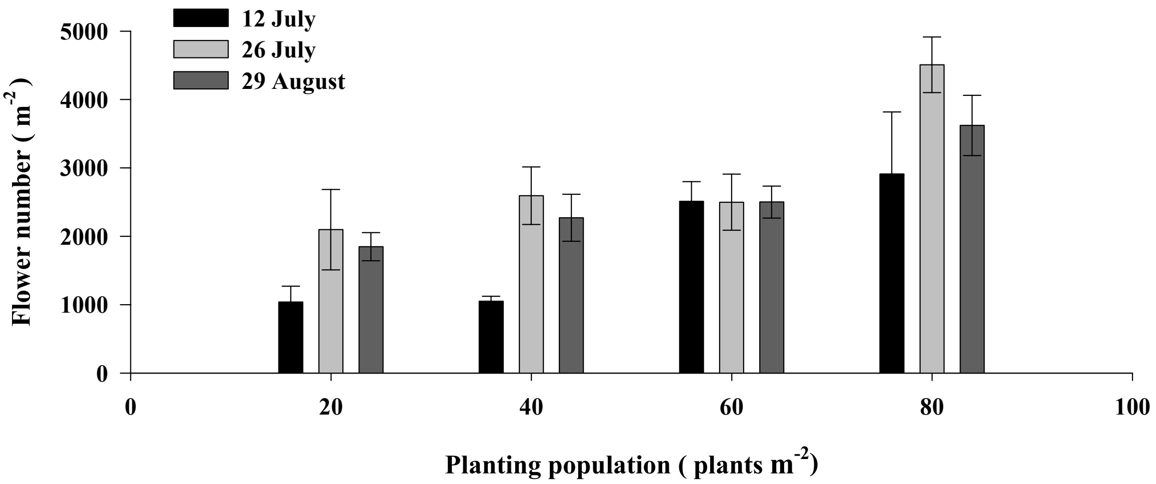



**Fig. 7.** *Flower number $m^{-2}$ at different planting densities on three dates, cultivar 981188-4, 2000.*

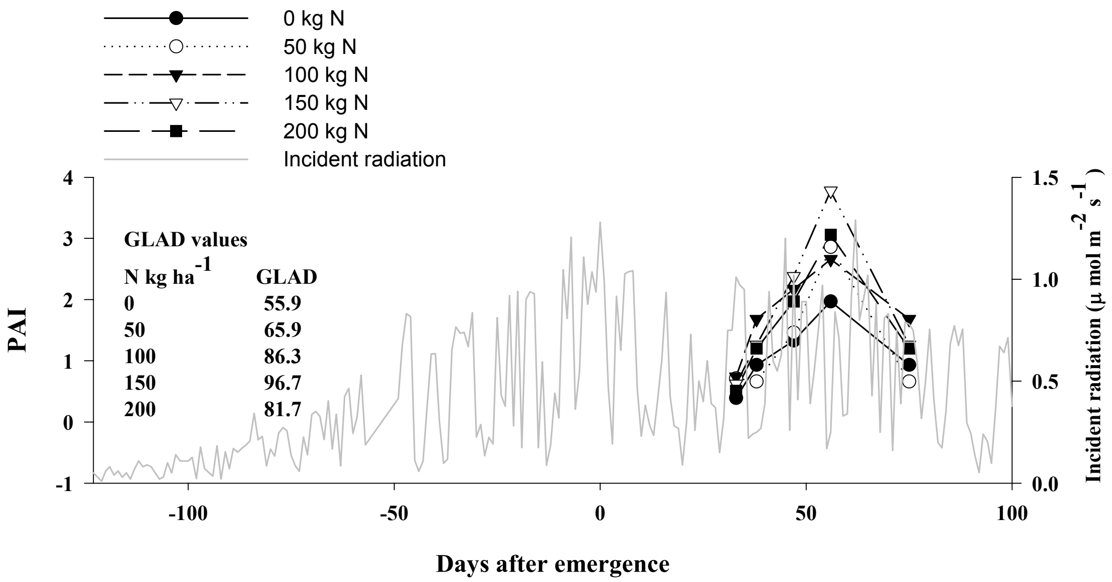



**Fig. 8.** *Photosynthetic area index (PAI) development through the growing season for* C. officinalis *at four planting densities (all plots supplied with 50 kg $ha^{-1}$ N), 2000.*

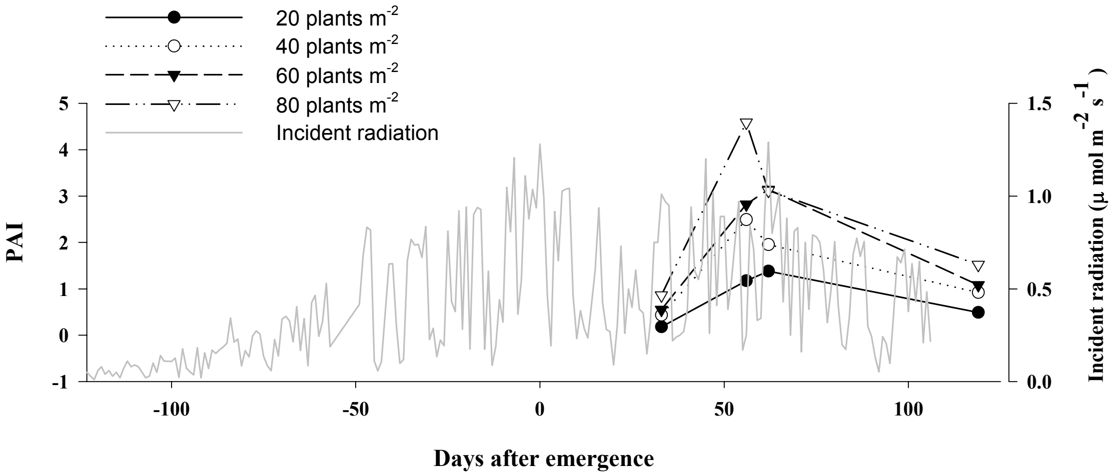



**Fig. 9.** *Photosynthetic area index (PAI) development through the growing season for* C. officinalis *at five nitrogen fertilisation levels (all plots sown at 60 plants $m^{-2}$), 2000.*

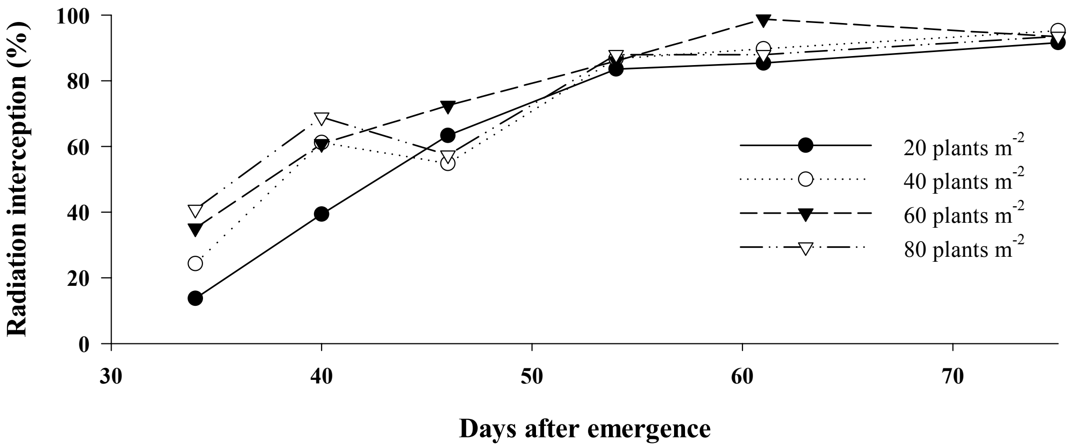



**Fig. 10.** *Radiation interception (%) at different planting densities,* C. officinalis, *2000.*

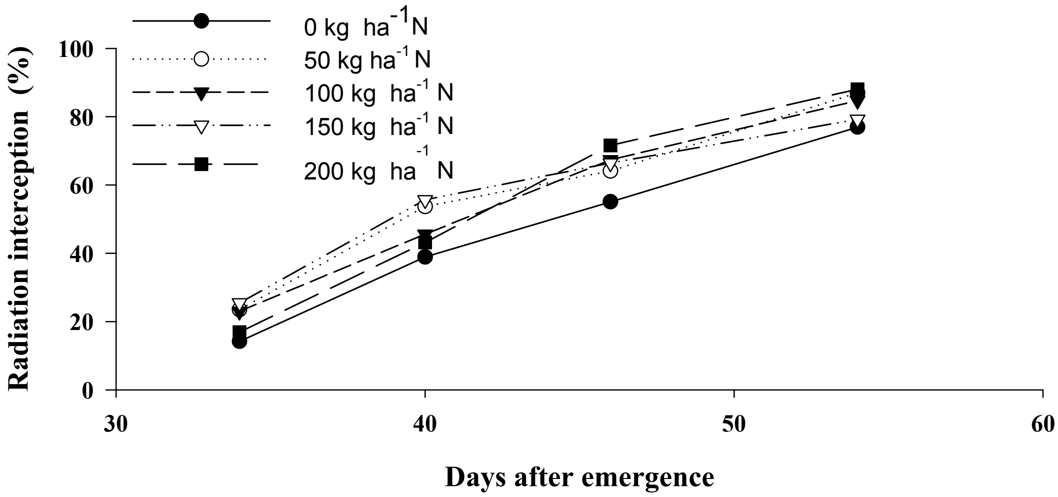



**Fig. 11.** *Radiation interception (%) at different rates of nitrogen fertiliser,* C officinalis, *2000.*

**Discussion**

Results from this study have demonstrated that *C. officinalis* is capable of up to 2.56 t $ha^{-1}$ seed yield under UK conditions. This represents a considerable increase over previously reported UK yields (Cromack *et al*., 1993) and indicates that the doubling of yield to 3 t $ha^{-1}$ predicted by Marvin (1999) is feasible under favourable agronomic and climatic conditions. This yield increase was attained by improved selection of cultivar, planting density and nitrogen application. Use of the new cultivar 981188-4 resulted in approximately 1 t $ha^{-1}$ increase over the older cultivar, cultivar HC.

Applications of nitrogen to cultivar 981188-4 up to 84 kg $ha^{-1}$ N, in an N-depleted soil, resulted in significant yield increases in the high yielding 2000 season. In the lower yielding 1998 and 1999 seasons, the crop appeared to be adequately supplied by soil residual N of 120-130 kg $ha^{-1}$, suggesting a crop N requirement from soil and fertiliser of 100 kg $ha^{-1}$ N for a 2.3 t $ha^{-1}$ crop. Crop N offtake in seed was about 25 kg $t^{-1}$, suggesting that a grower recommendation of 100-120 kg N $ha^{-1}$ would meet all crop needs. This N requirement is similar to other spring-sown oilseed crops in the UK such as oilseed rape (MAFF, 2001).

The selection of optimal planting density within the range evaluated in these experiments appears less critical to achieving high seed yields, due to the compensatory abilities of this indeterminate species. However, these effects were masked to some extent by plant population densities that were greater than the target densities in two of the experimental years. With the lowest plant density of 20 plants $m^{-2}$, biomass dry matter yield was significantly reduced. This treatment also gave the lowest seed yield and whilst these differences in yield were not significant, the result suggests a lower limit to the plant's compensatory abilities. Densities above 40 plants $m^{-2}$ offered little extra benefit in terms of increased seed yield or perhaps more notably competitive canopy development. Indeed, mutual shading of lower leaves and flower heads of *Calendula*, may have impeded yield formation. Other oilseeds, for example *Brassica napus,* gain most of their photoassimilates for oil production from pod photosynthesis rather than relocation of stem reserves (McWilliam *et al*., 1995, Mendham, 1995). Whilst insufficient evidence was gained in this experiment to determine the role, if any, of inflorescence photosynthesis in *Calendula* yield formation, it was noted that at high plant densities, more flowers were produced but yield was not enhanced, so yield per flower was reduced. Whether this was merely modified resource allocation (i.e. sink modification) or source limitation through mutual shading at high densities, should be further investigated. The early competitive ability of the *Calendula* crop is important for effective weed control. Herbicide options in the *Compositae* are limited and rely on residual herbicides, which require good soil conditions and moisture to work effectively (Cromack *et al*., 1997). Competitive canopies, in part provided by higher plant populations, can therefore be justified commercially for this objective.

Experiments by Cromack & Smith (1998) examined the relationships between densities from 10 - 120 plants $m^{-2}$ over two seasons. In one season, with a late maturing cultivar, there was no effect on seed yield but, in the second season, yields increased with density, although only up to 40 plants $m^{-2}$. Borm & van Dijk (1994) reported similar results, but Röbbelen *et al*. (1994) suggested responses up to 60 plants $m^{-2}$. Possible interactions between cultivars and density may explain these contrasting responses, but the data suggest that 40 plants $m^{-2}$ is an acceptable target using current, commercially available cultivars.

Assessments of flowers within the crop canopy showed that flower number $m^{-2}$ increased with planting density, despite some compensatory effects at lower densities from increased flower number/plant. Overall however, seed yield was unaffected by density, suggesting that flower number was not limiting yield expression.

At higher plant densities, maximum PAI was higher, although this brought little benefit in terms of enhanced radiation interception. The short growing season and late sowing date relative to the seasonal increase in incident radiation in the south of the UK appears to limit crop performance. Earlier sowing in mid-March might be beneficial if temperature conditions were favourable for crop growth. This suggests the need to develop more hardy genotypes. Alternatively, autumn sowing or dual cropping approaches might be appropriate.

**OBJECTIVE 2. TO IDENTIFY GENOTYPE × ENVIRONMENTAL INTERACTIONS TO AID THE SELECTION AND PRODUCTION OF THE MOST APPROPRIATE GENOTYPES FOR SOUTHERN ENGLAND, USING ASSESSMENT TECHNIQUES DEVELOPED FROM THE RESEARCH OUTLINED ABOVE**

**Materials and Methods**

*Trial sites*

Experiments were conducted in 1998, 1999 and 2000 on commercial farm sites in Devon, UK. Two experimental sites were used, Credition (1998) and Starcross (1999 & 2000). Both had free draining sandy-loam soils.

*Treatments and experiment design*

Ten cultivars (Table 4) of *C. officinalis* were randomised within each of 3 blocks.

**Table 4.** *Cultivars assessed in each year of the project. Cultivars in bold were common to all years.*

| 1998 | 1999 | 2000 |
| --- | --- | --- |
| **Hens and Chickens** | **Hens & Chickens** | **Hens and Chickens** |
| Pot Marigold | PRK98 | PRK99 |
| PRK97 | **165-7** | **931004** |
| **921108** | **277-9** | **277-9** |
| 90-7 | **921108** | **921108** |
| **931004** | **931004** | **165-7** |
| **277-9** | 971208 | 981188 |
| **165-7** | 971188 | 981208 |
| 883106 | 981617 | 981617 |
| 96-1 | 981638 | 981638 |

*Crop management and harvesting*

The plots were drilled with an Øyjord precision seed drill adjusted for a row width of 20cm. The seed rate was calculated to give a target plant population of 60 plants $m^{-2}$ by measuring the thousand seed weight for each cultivar, assuming an 80% germination rate and 60% field emergence. Plot length was 8.1 m. Drilling dates were 8 May 1988, 5 May 1999 and 3 May 2000. Phosphate, potash and sulphur fertilisers were applied across the site at a rate of 60 kg $ha^{-1}$ $P_2O_5$, 60 kg $ha^{-1}$ $K_2O$ and 21 kg $ha^{-1}$ S by hand. Nitrogen was applied by hand, as ammonium nitrate, to all plots at a rate of 50 kg $ha^{-1}$ N in the seedbed and again in mid-July. All plots were desiccated with Reglone (diquat) at 3 l $ha^{-1}$ and harvested using a Sampo Rosenlew 20/25 plot combine. Seed was collected in paper sacks and weighed. A sub sample of 100 g of seed was oven dried at 100 $^{o}$C for 16 h to determine dry matter content.

*Soil and plant measurements*

Date of 50% plant emergence was visually determined by assessing the percentage emergence on each plot on 2 or 3 dates. These data were averaged for each cultivar and the date of 50% emergence interpolated or extrapolated from these means. The same technique was used for estimating date of flowering (when 50% of plants had open flowers) and date of seed maturity (when 50 % of heads were brown). Percentage light interception was measured weekly until canopy closure using a Sunscan canopy analysis system.

Total above ground dry matter yield and partitioning into plant components were measured 2 days before crop desiccation. All the brown and green heads were removed from within a 1 $m^2$ quadrat. Plants were cut just above ground level using secateurs and removed. All harvested material was weighed fresh and a sub sample of 4 or 5 whole plants taken. This sub-sample was divided into leaves and stems. Each fraction was weighed fresh, dried for 18 h at 100 $^{o}$C and then weighed to determine the dry matter yields. The leaf fraction was too small and senesced to measure leaf area. The sample of brown heads was dispatched to CPRO for oil and calendic acid analysis.

*European-wide genotype x environment experiment*

Ten genotypes were successfully sown and harvested in replicated field experiments at two sites (the Netherlands and the UK) in 1998, at 6 sites (Lelystad (NL), Starcross (UK), Wageningen-1 (NL), Wageningen-

2 (NL), Jena (GER) and Bazierge (FR)) in 1999 and at 5 sites (Lelystad (NL), Starcross (UK), Wageningen (NL), Jena (GER) and Bazierge (FR)) in 2000. Experiment plots were assessed for a range of characters including seed yield and oil content and calendic acid content.

*Statistical analysis*

To allow examination of the interaction between genotype and environment, data for cultivars common to all years (five cultivars) were analysed by analysis of variance (ANOVA) in a multi-season analysis, using year as a factor. For each variate, two analyses were performed: one including all three years and one including data from the two years (1999 and 2000) when the experiment was at the same site. In addition, in 2000, a multi-site analysis was performed in a similar way using yield data from sites located in other EU states, together with data from Starcross.

**Results**

There was less than 1 week between drilling and 50% emergence of any cultivar in each year. Emergence took 13-16 days in 1998, 10-17 days in 1999 and all ten cultivars emerged 10 days after drilling in 2000. The duration from emergence to 50% flowering took between 47 and 55 days in 1998, between 46 and 51 days in 1999 and between 47 and 54 days in 2000. The time from emergence to 50% mature seed head stage took between 96 and 107 days in 1998 and between 92-93 days in 1999. No records were taken in 2000. In both 1998 and 1999, cultivar HC achieved 50% flowering 2-3 days later than the other cultivars common to all three years, yet achieved 50% mature flower heads slightly earlier than the others.

In 2000, HC was affected by lodging, with 50% of one plot lodged and 70% of another. Plant density was correlated with light interception and high plant populations consistently intercepted more light than low plant populations (data not presented).

The statistical significances of differences between the five cultivars (see Table 4) common to all years are shown in Table 5. In the analysis of data from three seasons, there was a significant effect of year in all parameters shown, but a significant difference between cultivars and a significant interaction between cultivar and year were only found in the case of combine-harvested yield. Data for all other parameters shown were obtained from smaller, quadrat samples.

In the analysis of data from the two seasons when the field experiments were at the same site, there were no significant cultivar-year interactions except in the case of calendic acid content in the extracted oil. Cultivar-year interactions are of particular interest because these indicate an interaction between genotype and environment. Where there is no interaction, the implication is that the performance of cultivars, relative to each other, was not influenced by environment.

Significant differences shown in Table 5 are illustrated in the following bar charts (Figs. 12-19). Figures 12, 15, 16 and 17 show differences between seasons. Differences in seed yield from combine harvesting followed a different pattern to differences between seasons in seed yield from quadrats. A notable difference between seasons in the dry weights of above-ground components is that, in 1998, there was a smaller proportion of seed heads and a greater proportion of stem relative to total biomass and seed yield, than in 1999 and 2000 (Fig. 15). In 1998, the weight of seed heads was only slightly greater than the seed yield, whereas in 1999 and 2000 weights of seed heads were several times greater than the yield, indicating that seed head ripening was much less uniform. Mature seed heads in good condition consist almost entirely of seed.

Season rankings for oil content of seed (Fig. 16) and calendic acid content of oil (Fig. 17) were the same, which would amplify the seasonal differences in calendic acid yield. In the analysis of data from the two seasons when the field experiments were at the same site, there were differences between cultivars in calendic acid content of oil (Fig. 18) and a significant interaction between cultivar and year (Fig. 19). Cultivar 921108 had a similar calendic acid content of oil in 1999 and 2000, whereas for the other four cultivars, values were lower in 1999 than in 2000. Cultivar 921108 had the highest calendic acid content of oil in 1999 and the second highest in 2000, but also had the lowest seed yield in the same years.

**Table 5.** *Statistical significance of differences between the five cultivars (see Table 4) common to all years.*

| | Statistical significance (NS = not significant; numbers indicate probability of no difference between cultivars) | | | | | |
|---|---|---|---|---|---|---|
| | 3 year analysis (1998 - 2000) | | | 2 year analysis (1999 - 2000) | | |
| Factor / Parameter | year | cultivar | yr.cv | year | cultivar | yr.cv |
| **Yield (combine)** | <0.001 | 0.024 | 0.011 | <0.001 | 0.011 | NS |
| **Yield (quadrat)** | 0.014 | NS | NS | 0.007 | 0.023 | NS |
| **Biomass per unit area** | | | | | | |
| Total above-ground biomass | 0.013 | NS (0.055) | NS | 0.021 | NS | NS |
| heads | <0.001 | NS | NS | 0.02 | NS | NS |
| stem | <0.001 | NS | NS | NS | NS | NS |
| leaf | <0.001 | NS | NS | 0.004 | NS | NS |
| **Oil content (% of seed dry wt)** | <0.001 | NS | NS | NS | NS | NS |
| **Calendic acid (% of oil)** | <0.001 | NS | NS | 0.005 | 0.002 | 0.016 |

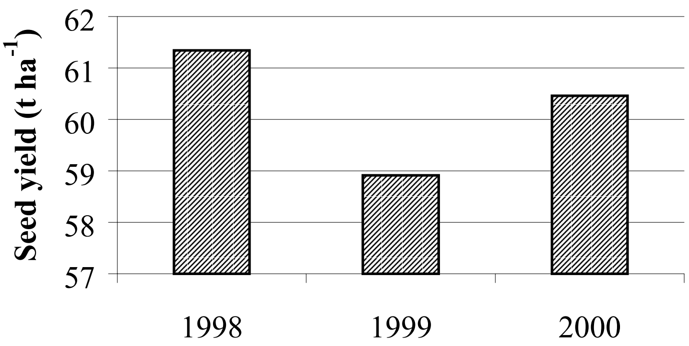



**Fig. 12.** *Seed yield at 9% moisture content, assessed using a combine harvester, means of five cultivars common to three seasons.*

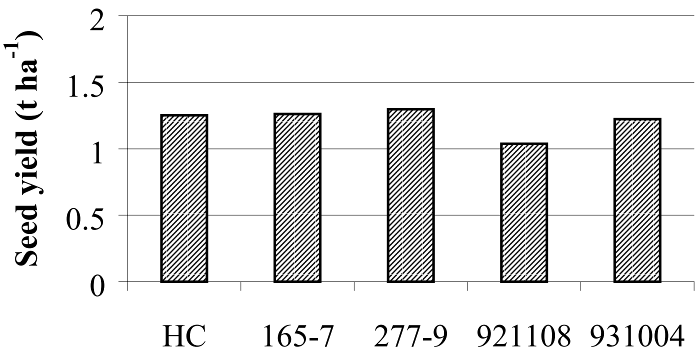



**Fig. 13.** *Seed yield at 9% moisture content, assessed using a combine harvester, means of three seasons for five cultivars.*

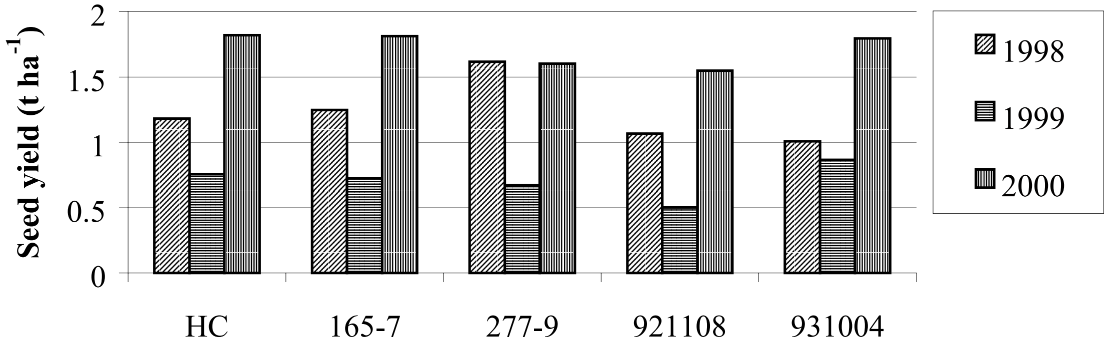



**Fig. 14.** *Seed yield at 9% moisture content, assessed using a combine harvester, for each of five cultivars in three seasons.*

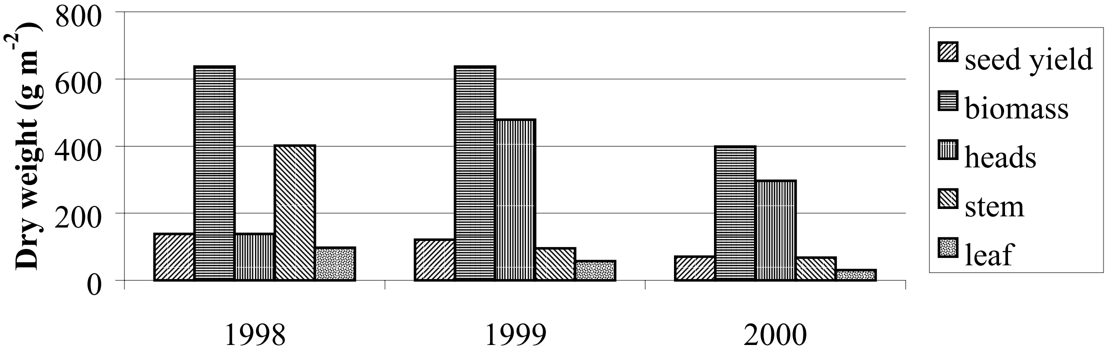



**Fig. 15.** *Components of yield at 100% dry matter (dm) (seed yield, above-ground biomass, seed heads, stem and leaf) at crop maturity, means of five cultivars common to three seasons.*

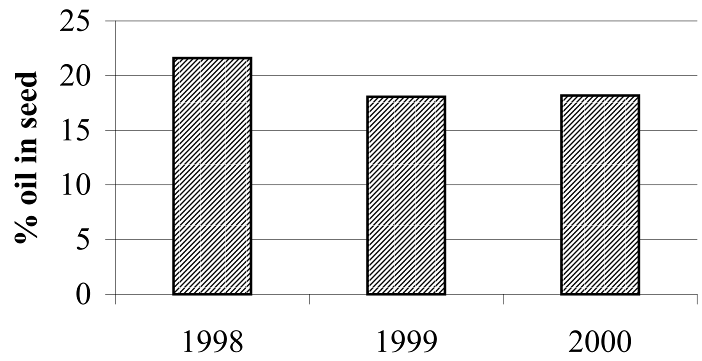



**Fig. 16.** *Oil content of seed (%), means of five cultivars common to three seasons.*

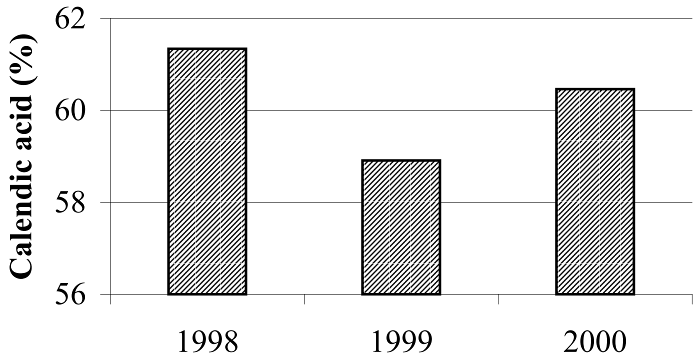



**Fig. 17.** *Calendic acid content expressed as % of oil, means of five cultivars common to three seasons.*

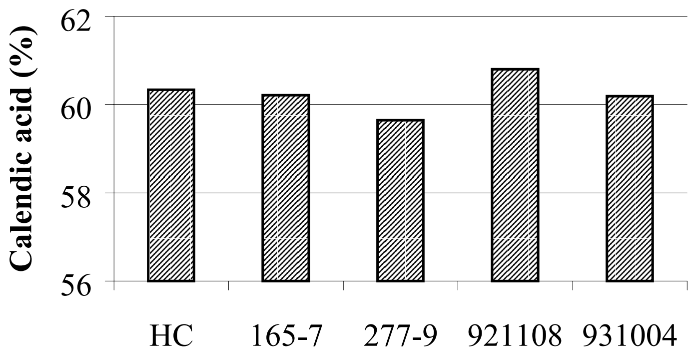



**Fig. 18.** *Calendic acid content expressed as % of oil, means of two seasons (1999 and 2000) for each of five cultivars.*

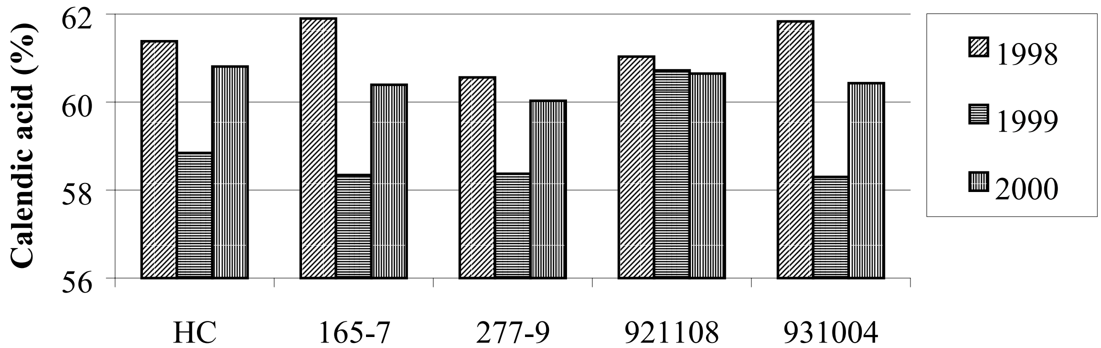



**Fig. 19.** *Calendic acid content expressed as % of oil, for each of five cultivars in three seasons.*

*European-wide genotype x environment experiment 1998*

Ten genotypes were sown both in the Netherlands and the UK. There were significant differences between the performance of individual genotypes and between sites in terms of seed yield, oil and calendic acid content. Seed yields ranged from 0.6 to 1.7 at the NL site and 0.4 to 1.6 t $ha^{-1}$ at the UK site, although overall yields were slightly higher at the UK site. The later maturing Pot Marigold yielded poorly at both the NL and UK sites, whilst the genotypes 277-9, 883106, 90-7 and PRK97 all performed well. Seed oil contents were consistently higher at the UK site, ranging from 20-22% oil, compared to 17-20% at the NL site. This result is likely to reflect local growing conditions and demonstrates the importance of studying interactions between genotype and environment for the identification of site effects and cultivars capable of consistent performance across a range of sites. Comparing the relative oil content across the two sites revealed that 165-7, 277-9 performed better than most genotypes across both sites. 96-1 and 931004 performed well at the NL site. Calendic acid contents were also consistently higher at the UK site, ranging from 60-64% compared to 57-63% at the NL site. Comparing relative performance at the two sites clearly revealed two genotypes, 90-7 and 883106, producing oil with high calendic acid contents.

*European-wide genotype x environment experiment 1999*

Mean seed yield (cultivar means) ranged from 1272 up to 1605 kg ha$^{-1}$ across all six sites, with mean site yield ranging from only 862 kg ha$^{-1}$ at Bazierge in southern France to 1900 kg ha$^{-1}$ at Wageningen-2. The highest mean seed yield was produced by PRK98, one of the cultivars being grown by farmers in collaboration with Cebeco. Seed oil content was low in PRK98 at 7.9%, but reached 21.7% in line 971188. Mean seed oil yield across all six sites and cultivars was 290 kg ha$^{-1}$, but the best line, 971188 achieved 332 kg ha$^{-1}$ across all sites and 472 kg ha$^{-1}$ at one site, Wageningen-2.

The performance improvements of new genotypes, in terms of seed and oil yield ha$^{-1}$, compared to the old cultivar HC, demonstrate the plant breeding achievements within the project. The genotype x environment experiments have also provided a valuable test of the potential of *Calendula* at contrasting European sites. Whilst there was significant variation between locations, the southern European site performing poorly, the comparison of genotypes across sites showed good adaptation of the improved lines across all sites.

*European-wide genotype x environment experiment 2000*

Mean site yield ranged from only 826 kg ha$^{-1}$ at Bazierge in southern France to 2573 kg ha$^{-1}$ at Jena. The highest mean seed yield was produced by PRK99, also the highest yielding cultivar in 1999. Seed oil content was low in PRK99 at 16.4%, but reached 21.7% in line 971188. Mean seed oil yield across all five sites and cultivars was 292 kg ha$^{-1}$, but the best line, 971188 achieved 355 kg ha$^{-1}$ across all sites and 590 kg ha$^{-1}$ at Jena. The adaptability of genetic material to a range of environments is a highly desirable character, as it reduces variation in performance and therefore financial risk for farmers and security of supply for oil buyers. The relatively high yield performance of cultivar HC at the Jena site in 2000 was surprising, as it matched that of improved lines. However, the overall results clearly demonstrated the benefits of the improved lines over the old type, in terms of yield stability.

There was significant variation between sites in crop performance: the southern European site again performed poorly.

Means of seed yield and oil content and calendic acid content of oil are tabulated in Tables 6 to 8 below. Multi-site analysis of data (ANOVA) from five sites in 2000 showed that there were significant interactions between site and cultivar for seed yield ($P < 0.001$) and calendic acid content of oil ($P < 0.001$), but not for seed oil content. This demonstrates a genotype $\times$ environment interaction for seed yield and calendic acid content of oil, but shows that in this case there was no evidence that cultivar differences in seed oil content were affected by environment.

**Table 6.** *Seed yield (kg ha$^{-1}$) at 0% moisture content, assessed using a combine harvester, for each of ten cultivars at five sites in 2000. Numbers in bold and italics indicate the cultivars with the greatest and smallest yields respectively for each site.*

| | Cultivar | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **HC** | **PRK99** | **931004** | **277-9** | **921108** | **165-7** | **981188** | **981208** | **981617** | **981638** |
| Lelystad | *1273* | 1574 | 1391 | 1827 | 1549 | 1389 | **1844** | 1565 | 1276 | 1475 |
| Starcross | 1656 | 1640 | 1632 | 1457 | *1408* | 1648 | 1479 | 1495 | **1743** | 1562 |
| Wageningen | 780 | 976 | 944 | **1446** | 883 | 791 | 1201 | 1193 | *699* | 1197 |
| Jena | 2717 | 2676 | 2718 | *2116* | 2355 | **2744** | 2467 | 2608 | 2739 | 2587 |
| Bazierge | *396* | **1131** | 1035 | 953 | 936 | 900 | 837 | 634 | 751 | 688 |
| Mean | *1364.4* | **1599.4** | 1544.0 | 1559.8 | 1426.2 | 1494.4 | 1565.6 | 1499.0 | 1441.6 | 1501.8 |

| SED (18 d.f.) | | | | |
|---|---|---|---|---|
| | Lelystad | 89.0 | Jena | 203.2 |
| | Starcross | 103.7 | Bazierge | 178.6 |
| | Wageningen | 177.2 | | |

**Table 7.** *Oil content of seed (%), for each of ten cultivars at five sites in 2000. Numbers in bold and italics indicate the cultivars with the greatest and smallest yields respectively for each site.*

| | Cultivar | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **HC** | **PRK99** | **931004** | **277-9** | **921108** | **165-7** | **981188** | **981208** | **981617** | **981638** |
| Lelystad | 19.96 | *18.47* | 19.94 | 21.41 | 21.81 | 19.14 | **23.54** | 22.04 | 22.71 | 22.48 |
| Starcross | 18.92 | *14.57* | 16.30 | 18.30 | 18.21 | 19.14 | 20.56 | 19.37 | 19.80 | **21.27** |
| Wageningen | 19.88 | *16.60* | 18.17 | 19.02 | 19.78 | 17.83 | 21.14 | 18.43 | **21.33** | 20.96 |
| Jena | 22.92 | *18.92* | 20.49 | 22.73 | 20.48 | 21.14 | **23.91** | 20.66 | 21.83 | 22.42 |
| Bazierge | 15.38 | *13.36* | 14.02 | 16.48 | 16.94 | 15.32 | **19.27** | 15.86 | 15.66 | 18.40 |
| Mean | 19.41 | *16.38* | 17.78 | 19.59 | 19.44 | 18.51 | **21.68** | 19.27 | 20.27 | 21.11 |
| SED (18 d.f.) | | Lelystad | 0.793 | | Jena | 1.046 | | | | |
| | | Starcross | 1.247 | | Bazierge | 1.027 | | | | |
| | | Wageningen | 1.009 | | | | | | | |

**Table 8.** *Calendic acid content expressed as % of oil, for each of ten cultivars at five sites in 2000. Numbers in bold and italics indicate the cultivars with the greatest and smallest yields respectively for each site.*

| | Cultivar | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **HC** | **PRK99** | **931004** | **277-9** | **921108** | **165-7** | **981188** | **981208** | **981617** | **981638** |
| Lelystad | 61.1 | 62.7 | *60.3* | 61.0 | 61.9 | 61.9 | **66.2** | 61.5 | 60.4 | 63.5 |
| Starcross | 60.8 | 62.7 | 60.4 | *60.0* | 60.6 | 60.4 | **63.5** | 60.2 | 61.4 | 61.9 |
| Wageningen | 60.5 | 62.5 | 60.8 | *59.8* | 61.3 | 61.4 | **65.2** | 60.2 | 60.6 | 63.8 |
| Jena | 61.7 | 63.2 | 61.4 | *61.0* | 62.4 | 62.3 | **64.9** | 61.5 | 62.6 | 64.6 |
| Bazierge | 55.9 | **58.7** | 56.0 | 55.4 | 56.8 | 56.1 | 58.7 | *55.3* | 57.3 | 57.0 |
| Mean | 60.0 | 62.0 | 59.8 | *59.4* | 60.6 | 60.4 | **63.7** | 59.8 | 60.4 | 62.2 |
| SED (18 d.f.) | | Lelystad | 0.31 | | Jena | 0.40 | | | | |
| | | Starcross | 0.40 | | Bazierge | 0.74 | | | | |
| | | Wageningen | 0.39 | | | | | | | |

**Discussion**

Differing methodology for seed yield assessment (combine harvesting of a whole plot or hand harvesting of a 1 $m^2$ quadrat) gave different results. Smaller quadrat samples could have been subject to greater spatial variability than the large samples. However, the combine harvesting method is prone to other sources of error that hand harvesting is not. For example, different cultivars had different phenology, possibly resulting in differences in the degree of maturity at harvest and this could influence the fragility of the seed head and the extent of seed loss during combine harvesting. Combine harvesting is more representative of commercial practice, but it is not possible to say whether the cultivar differences and year-cultivar interactions would have been observed if harvest timing had been tailored to individual cultivars. Unfortunately, there is not a satisfactory solution to this problem in field experiments because harvesting each cultivar at a different time would confound cultivar comparisons with differences in weather conditions at harvest.

Mature seed heads in good condition consist almost entirely of seed, but in 1999 and 2000 weights of seed heads were several times greater than the seed yield, indicating that seed head ripening was not uniform (see Fig. 15). These results suggest that the potential yield is much greater than the actual yields obtained in these experiments. Greater uniformity of maturity should be a target for breeders.

Crop performance at the southern European site was poor, suggesting that although *Calendula* is of Mediterranean origin, environmental factors do not favour its commercialisation in southern Europe.

The results clearly demonstrated the benefits of the improved lines over the old type, in terms of yield stability, with good yields across several sites with differing environments.

**ADDITIONAL MILESTONE-EXAMINE THE OPPORTUNITIES FOR BIENNIAL CROPPING IN CALENDULA**

This additional milestone was agreed in 2000. *Calendula,* when grown in a non-cropping environment, is capable of performing as a biennial. If this could be demonstrated in field crops, it could be of agricultural value because it would reduce the input costs and husbandry requirements of the crop whilst (potentially) maintaining yield.

A replicated field experiment was conducted in south-west England, at Starcross, examining the biennial nature of *C. officinalis*. Individual plot size was 13.3 m x 2 m. Seeds were sown on 3 May 2000 using an Øyjard seed drill at a 20 cm row spacing. Fertiliser was applied at application rates of 50 kg $ha^{-1}$ N, 50 kg $ha^{-1}$ $P_2O_5$ and 180 kg $ha^{-1}$ $K_2O$. Pendimethalin was applied at 2.5 l $ha^{-1}$ on 4 May 2000 for weed control. Sulphur was applied to the crop as a spray at 10 kg $ha^{-1}$ in 250 l $ha^{-1}$ water on 30 June 2000. All plots were harvested on 11 September 2000 with a Wintersteiger combine harvester. After harvest, plant counts were made in 1 $m^2$ quadrats and further plant counts were made in the same quadrats in January 2001 (Table 9).

**Table 9.** *Plant density, 04 October 2000 and 24 January 2001.*

| | Plant density | |
|---|---|---|
| Plot number | 04 Oct 2000 | 24 Jan 2001 |
| Mean of 20 plots | 66.85 | 6.85 |

In spring 2001, no over-wintering plants remained in the plots. It is concluded that *C. officinalis* is unlikely to be successful as a biennial crop in the UK.

**CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK**

Optimum plant density for seed yield was less than 40 plants $m^{-2}$; at lower densities individual plants were larger, but did not produce comparable dry matter yields per unit ground area as higher planting density treatments. Optimum crop N requirement for biological and economic yield was 100 kg N $ha^{-1}$. Fertiliser N requirement would depend on available soil N, related to soil type and previous cropping, but is low compared to many other crops.

The short growing season and late sowing date relative to the seasonal increase in incident radiation in the south of the UK appears to limit crop performance. Earlier sowing in mid-March might be beneficial if temperature conditions were favourable for crop growth. This suggests the need to develop more hardy genotypes. Alternatively, autumn sowing or dual cropping approaches might be appropriate. However, no further work on biennial cropping is recommended with currently-available cultivars.

Whilst insufficient evidence was gained in this experiment to determine the role, if any, of inflorescence photosynthesis in *Calendula* yield formation, it was noted that at high plant densities more flowers were produced, but yield was not enhanced, so yield per flower was decreased. Whether this was merely modified resource allocation (i.e. sink modification) or source limitation through mutual shading at high densities, could be further investigated.

Interaction between genotype and environment was observed, but improved lines showed good adaptation to differing environments. However, environmental factors do not favour commercialisation of *Calendula* in southern Europe.

Alkyd paints with less volatile organic compounds (VOC) and non-fogging polyurethane foams (PUF) can be produced with calendula oil as raw material and their use would reduce VOC-emissions. However, with current calendula oil yield and processing efficiency, the cost price of the oil is too high for competition with petrochemical raw materials for high emission paints and PUF. Further work could be aimed at reducing the cost price by increasing oil yield through *Calendula* breeding and agronomy.

It is suggested that breeding priorities should be directed towards winter hardiness, to allow earlier sowing and greater light interception, and uniformity of maturity or better seed retention (less seed shedding).

A growers' guide has been produced as part of this project and a copy is attached to this report.

A summary of the progress made in the EC project, of which the work reported here formed a part, is given in theExecutive Summary. A further extract from the same report (van Loo, 2002) is given in conclusion, relating to future actions.

> As for the future actions after this project, part of the consortium of this project (namely PRI, CEBECO, DSM, Van Wijhe and DeSmet) are now investigating the possibilities of starting an calendula oil production chain with primary production and extraction in Marocco as the agricultural production in the current EU-countries is still to expensive. The oil will be used by current tung users (varnish and standoil industry) and by the paint manufacturers (DSM and Van Wijhe) from this product, after it has been shown that the cost price of the Calendula oil from Marocco is really low enough. The paint

manufacturers in this project continue to improve the reactive diluent/resin formulation in order to reach a paint product quality that is comparable to standard alkyd paints. The polyol producer from the project (KAJO) is currently investigating the possibilities of developing the new polyurethane foam developed in this project – together with a large polyurethane factory.

Considering, the market potential of Calendula is almost proven. Yet, a few years of product and market development are needed before we really know whether Calendula oil will be a success.

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