

## **Towards domestication of *Dimorphotheca pluvialis***

Studies on the genetic improvement of a potential  
oilseed crop for industrial applications

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## **Towards domestication of *Dimorphotheca pluvialis***

Studies on the genetic improvement of a potential  
oilseed crop for industrial applications

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**Abstract:**

The interest in arable crops for non-food use has increased substantially in the past few decades. In this thesis, research is described on aspects of domestication of *Dimorphotheca pluvialis* (L.) Moench, a potential oilseed crop for industrial applications. Seeds of this species contain oil with ca 60-65% dimorphecolic acid ( $\Delta^9$ -hydroxy,10t,12t-octadecadienoic acid), an interesting fatty acid because of its unique structure. The species is well adapted to the climatic conditions of north-west Europe, and fits well in a crop rotation system with annual crops. Emphasis is laid on genetic improvement of yield components. The currently available populations of *D. pluvialis* show considerable variation for morphological and agronomic traits, which is undesirable in modern agriculture. The species is predominantly outcrossing, and pollination is established mainly by insects. The average oil content of the seeds was rather low (21%) but can substantially be increased by means of mass selection; an average oil content of at least 30% seems feasible. Synchronisation of flowering between plants can quickly be achieved by mass selection for onset of flowering. Synchronisation of flowering within plants (duration of flowering) showed low to moderate heritability values, indicating that for improvement of this trait methods other than mass selection should be considered. Mass selection for plant architecture was very effective, even at a low selection pressure. Seed yields were relatively low and rather erratic (500-1800 kg/ha) due to seed shattering. Therefore, attention should be directed towards improvement of seed retention.

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## Stellingen

1. Het verdient aanbeveling bij subsidiëring van onderzoeksprojecten met betrekking tot industriële gewassen de projectduur te baseren op wetenschappelijke inzichten in plaats van op politieke argumenten.
2. Bij domesticatie staat het natuurlijk ecosysteem onder invloed van het menselijk ecosysteem.
3. Het onderzoek naar de productie van nieuwe plantaardige grondstoffen dient te worden afgestemd op wensen vanuit de industrie.
4. Agrificatie-onderzoek waarbij alle schakels van de productieketen gelijktijdig worden onderzocht is weliswaar wetenschappelijk interessant maar vergroot de financiële risico's.
5. Bij gebruik van medicinale preparaten op basis van 'natuurlijke plantaardige grondstoffen' is de algemene veronderstelling 'baat het niet dan schaadt het niet' bedenkelijk, gezien het feit dat veel plantensoorten voor de mens giftige stoffen produceren.
6. Flexibiliteit met betrekking tot arbeid blijkt in de praktijk een rekbaar begrip; door werkgevers wordt het doorgaans anders geïnterpreteerd dan door werknemers.
7. Het opleggen en ten uitvoer brengen van de doodstraf is niet wezenlijk verschillend van moord met voorbedachte rade.
8. Om logistiek-economische redenen verdient het aanbeveling bij de veredeling van grootfruit te streven naar vierkante vruchtvormen.
9. De vermelding 'dermatologisch getest' op cosmetica is volkomen betekenisloos indien niet ook het testresultaat wordt vermeld.
10. Het door hondenbezitters veroorzaakte 'hondenpoepprobleem' wordt doorgaans slechts door niet-hondenbezitters als zodanig erkend.
11. Aangezien stress een negatieve invloed heeft op het geheugen<sup>7)</sup>, dienen stressvolle omstandigheden tijdens examens te worden vermeden.

<sup>7)</sup> Neurobioloog Paul Luiten, Intermediair 11, maart 2000.

Stellingen behorende bij het proefschrift "Towards domestication of *Dimorphotheca pluvialis* - Studies on the genetic improvement of a potential oilseed crop for industrial applications"

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*“To try something is not a guarantee for success,  
but not to try it is a guaranteed failure”*

(Bill Clinton, 2000)

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## Chapter 1

# **Domestication of oilseed crops for industrial applications, with particular reference to *Dimorphotheca pluvialis***

## **Domestication**

More than 99% of his presence on earth as a distinct species, man acquired food by hunting and gathering. Cultivation of plants started only about 10.000 years ago (Ucko and Dimbley 1969). At some time, intuitively the best plants were selected and used for the next planting. From that time on, the domestication of crop plants set in (Harlan 1975). Domestication is an evolutionary process operating under the influence of human activities, and regards the genetic response of the plants that are cultivated. It should not be confused with cultivation, which deals with conducting activities involved in caring for a plant, such as soil preparation, weeding, pruning, protecting, watering and manuring. It is very well possible to cultivate wild plants, and cultivated plants are not necessarily domesticated (Harlan 1975, Zeven and De Wet 1982).

In the wild, natural forces tend to select for characteristics favouring plant survival and reproduction. In addition, man started to select plants which are best adapted to human requirements. For some traits, e.g. those involving adaptation to climatological circumstances, natural and artificial (human) selection forces may work in the same direction and reinforce each other. For many other characteristics however, both selection forces may act in different or even opposite directions. The wild ancestors of wheat (*Triticum* spp.) for instance, have brittle ears from which seeds shed easily. This characteristic enhances seed dispersal, favouring survival of the species, but is highly undesirable to humans requiring reliable and constant yields. Human selection for less brittle ears has resulted in improvement of seed retention to an extent that modern wheat cultivars have become completely dependent on man for seed dispersal. Wheat can now be regarded fully domesticated for this feature (Zohary and Hopf 1993).

The changes brought about by the evolution from wild species to a modern domesticated crop can be quite drastic. Sometimes modern cultivars hardly resemble their wild ancestors. As was already noted by both Darwin (1859) and De Candolle (1886), the parts of the plant that were most valued by man generally show the greatest morphological alterations. Within some species, selection for different uses has resulted in extreme differences in plant appearance. For example, broccoli, cauliflower, Brussels sprouts, head cabbages, Chinese kale, curly kale and kohlrabi hardly resemble one another, although they all originate from *Brassica oleracea* L. (Hodgkin 1995).

The history of domestication of various crops shows considerable variation, and is certainly not something confined to the distant past. Archaeological research indicates that the domestication of wheat (*Triticum* spp.) and barley



(*Hordeum vulgare* L.), started as early as 8.000 BC., but the domestication of kiwi-fruit (*Actinidia* spp.) less than 100 years ago (Smartt and Simmonds 1995). Even today or in the future, man may start to domesticate wild species to exploit nature's potentials.

### **Oilseed crops for industrial applications**

Although early domestication of plant species most likely involved food and forage crops (Zeven and De Wet 1982), the use of plants for non-food purposes is probably almost just as old. In ancient times, non-food plants were used to produce e.g. construction materials, utensils/tools, dyes, poisons, drugs, medicines and fuel. In the course of time, many new industrial products based on agricultural materials were developed, such as paper, textiles, paints, plastics, lubricants, adhesives, surfactants (soaps and detergents) and rubber. The use of agricultural products in industry decreased in the 18th and 19th century, when an increasing knowledge of chemical processes resulted in the discovery of pathways for synthetic production from inorganic or mineral oil based raw materials (Princen 1982). The interest for the use of agricultural products in industry received a new impulse in 1957, when the United States Department of Agriculture (USDA) started an extensive exploratory chemical screening and utilisation research programme on new crops as sources of industrial oils, fibres, gums, natural rubber, carbohydrates, proteins and amino acids, substances with antitumor and pesticidal activity, and chemical intermediates. The reasons why this programme was initiated, were to alleviate the problems related to the overproduction of the major food crops in the USA, and to provide new opportunities for the chemical industry to use domestically produced raw materials instead of mineral oil based or imported commodities. Special attention was given to oilseed crops, and over a period of approximately 20 years, about 7.000 plant species from all over the world were screened for oil content, oil constitution, and potential as future crop. Though this was only a small fraction of the total number of known seed-bearing plant species, the programme led to the discovery of more than 75 new fatty acids (CAST 1984, Princen 1982 and 1983, Princen and Rothfus 1984).

Vegetable oils usually consist of a mixture of triacylglycerols (triglycerides). A triacylglycerol (Figure 1.1) is a glycerol molecule with different or identical fatty acids esterified to its three OH-positions. The fatty acids of vegetable oils consist of a hydrocarbon chain with a terminal carboxylate group. The number of carbon atoms is generally even, typically between 12 and 24. The 16- and 18-carbon fatty acids are most common (Stryer 1980). The carbon chains of different fatty acids do not only show variation in length, but may also be unsaturated at different (sometimes conjugated) positions, or have specific functional groups as hydroxy, keto or epoxy groups. Also (conjugated) combinations of (poly-)unsaturation with functional groups, and even branched or cyclic structures have been found (Stymne 1992, Badami and Patil 1981).

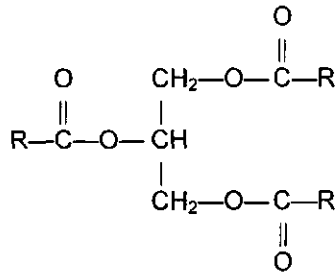


Figure 1.1. Molecular structure of a triacylglycerol. R = fatty acid acyl chain.

Fatty acids have systematic names based on the Greek word for the number of carbon atoms present. When present, however, fatty acids are usually referred to by their trivial names, which are often derived from the source of oil from which the fatty acid was originally isolated. Palmitic acid and oleic acid, for example, are the principal fatty acids of palm oil and olive oil, respectively, although these fatty acids are also found in other oil crops. In the literature, frequently shorthand designations are used to describe fatty acids. Though there are several ways of notation, in general, their basis is formed by two figures joined by a colon. The first figure is often preceded by a capital C and indicates the number of carbon atoms. The second figure, following the colon, indicates the number of double bonds. The position of the double bonds is usually counted from the carboxyl end, and is indicated by the number of the first unsaturated carbon atom, preceded by the symbol  $\Delta$  or  $\delta$ . Also, often the *cis*- or *trans*-orientation (sometimes abbreviated to *c*- and *t*-) of the double bonds is mentioned. The type and position of functional groups are usually presented before the carbon chain length (Äpplqvist 1989, Stryer 1980).

Each fatty acid has unique chemical and physical properties, and oil quality is largely determined by its fatty acid composition. Both oils with specific fatty acid compositions and oils with large quantities of one specific fatty acid may be of interest to the industry. Today, over 300 different fatty acids have been described, and the potential number of vegetable oils with different fatty acid composition is manifold (Stymne 1992). This variation, combined with a large number of possible chemical modifications, make vegetable oils/fatty acids versatile raw materials for industrial applications. Some interesting fatty acids and their vegetable sources are presented in Table 1.1 (Äpplqvist 1989, Hirsinger 1989, Lühs and Friedt 1994, Meier zu Beerentrop and Röbbelen 1987, Stymne 1992). Many 'unusual' oil types are restricted to one plant family or genus. Most often, these species have not previously been used for cultivation and show 'wild' characteristics as indeterminate growth, unsynchronised flowering, seed shattering or production of toxic by-products. For successful commercial production of these oils, wild plants need to be transformed into manageable crops. In other words, these species need to be domesticated.

Table 1.1. Fatty acids with potential use in oleochemical industry, with some vegetable sources.

Trivial fatty acid name	Short notation <sup>1)</sup>	Source(s)
Caprylic	C8:0	<i>Cuphea cyanea</i> Moç. & Sessé
Capric	C10:0	<i>Cuphea leptopoda</i> Hemsl.
Lauric	C12:0	coconut ( <i>Cocos nucifera</i> L.), oil palm ( <i>Elaeis guineensis</i> Jacq.), <i>Cuphea laminuligera</i> Koehne
Myristic	C14:0	coconut, oil palm, <i>Cuphea aequipetala</i> Car.
Palmytic	C16:0	oil palm
Petroselinic	C18:1 <sup>Δ6c</sup>	Coriander ( <i>Coriandrum sativum</i> L.), fennel ( <i>Foeniculum vulgare</i> Mill.), dill ( <i>Anethum graveolens</i> L.)
Oleic	C18:1 <sup>Δ9c</sup>	rapeseed ( <i>Brassica napus</i> L.), sunflower ( <i>Helianthus annuus</i> L.), oil palm, <i>Euphorbia lathyris</i> L.
Linoleic	C18:2 <sup>Δ9c, Δ12c</sup>	soybean ( <i>Glycine max</i> (L.) Merrill.), cotton ( <i>Gossypium</i> spp.)
γ-Linolenic	C18:3 <sup>Δ6c, Δ9c, Δ12c</sup>	<i>Borago officinalis</i> L., <i>Ribes</i> spp.
α-Linolenic	C18:3 <sup>Δ9c, Δ12c, Δ15c</sup>	linseed ( <i>Linum usitatissimum</i> L.)
Calendic	C18:3 <sup>Δ8t, Δ10t, Δ12c</sup>	<i>Calendula officinalis</i> L.
α-Eleostearic	C18:3 <sup>Δ9c, Δ11t, Δ13t</sup>	tung ( <i>Aleuritis fordii</i> Hemsl.), <i>Centranthus macrosiphon</i> Boiss.
α-Parinaric	C18:4 <sup>Δ9c, Δ11t, Δ13t, Δ15c</sup>	<i>Impatiens balsamina</i> L.
Crepenynic	Δ12-acetylenic-C18:1 <sup>Δ9c</sup>	<i>Crepis alpina</i> L.
Ricinoleic	Δ12-hydroxy-C18:1 <sup>Δ9c</sup>	castor ( <i>Ricinus communis</i> L.)
Dimorphecolic	Δ9-hydroxy-C18:2 <sup>Δ10t, Δ12t</sup>	<i>Dimorphotheca</i> spp., <i>Osteospermum ecklonis</i> (DC.) Norl.
Densipolic	Δ12-hydroxy-C18:2 <sup>Δ9c, Δ15c</sup>	<i>Lesquerella densipila</i> Rollins
Vernolic	Δ12-epoxy-C18:1 <sup>Δ9c</sup>	<i>Euphorbia lagascae</i> Spreng., <i>Vernonia</i> spp.
cis-5-Eicosenoic <sup>2)</sup>	C20:1 <sup>Δ5c</sup>	<i>Limnanthes</i> spp.
cis-11-Eicosenoic <sup>2)</sup>	C20:1 <sup>Δ11c</sup>	Brassicaceae, jojoba ( <i>Simmondsia chinensis</i> (Link) Schneider)
Lesquerolic	Δ14-hydroxy-C20:1 <sup>Δ11c</sup>	<i>Lesquerella</i> spp.
Auricolic	Δ14-hydroxy-C20:2 <sup>Δ11c, Δ17c</sup>	<i>Lesquerella auriculata</i> (Engelm. & Gray) Watts
Erucic	C22:1 <sup>Δ13c</sup>	<i>Crambe abyssinica</i> (Hochst.) ex. Fries, rapeseed
cis-cis-5,13-Docosadienoic <sup>2)</sup>	C22:2 <sup>Δ5c, Δ13c</sup>	<i>Limnanthes</i> spp.
Nervonic	C24:1 <sup>Δ15c</sup>	<i>Lunaria annua</i> L.

<sup>1)</sup> For an explanation of the short notation, see text.<sup>2)</sup> Systematic name, no known trivial name

## Modern domestication strategies

The domestication and development of each new crop requires a unique path dependent upon the specific characters of the plant species, history of the crop, and its potential uses. Nonetheless, some generalisations can be made about the steps involved. The U.S. Department of Agriculture (USDA) described a model comprising seven stages: germplasm collection, germplasm evaluation, chemical and utilisation studies, agronomic evaluation, crop breeding, production and processing scale-up, and commercialisation (CAST 1984). Van Soest (1994) underlined the importance of an early interaction between agriculture and industry, and described a model with four stages, each including an agricultural as well as an industrial contribution. This concept has been used in the new industrial crops research in the Netherlands in the past decade. The latter concept has been slightly modified and was subsequently called the '4-ex' model, named after its four stages: exploration, examination, expansion and exploitation. This model, including an approximate timetable, is presented in Table 1.2, and the stages are described below. To some extent the stages are sequential, but considerable overlap in time exists.

### *Stage 1. Exploration*

In the exploration stage, germplasm is collected and classified. Emphasis should be laid on collecting a broadly diversified array of seed samples, well representing the genepool of the species of interest. If germplasm is readily available and accessible from genebanks and botanical gardens for instance, this stage may take only a few years. Much more time may be needed if germplasm has to be collected, particularly if the species is widely dispersed or located in geographically or politically inaccessible areas. The newly obtained seed stocks usually need to be multiplied, which may take several generations of cultivation. When sufficient seed is available, evaluation of the collection can start in a field trial with small plots. This will give a first impression of agronomic, adaptive and phenological traits of a potential oil crop. Seeds are screened for principal constituents, and the most important chemical and physical properties of the oil and seed meal are determined.

### *Stage 2. Examination*

In this stage, the crop is grown in field trials at a variety of locations to assess its performance under a wide range of environments. Preferably, a large collection of seed samples, well representing the genepool, should be used, to determine the variation for relevant crop characteristics such as crop development, susceptibility to pests and diseases and seed yield. Cultural practices and harvesting methods are evaluated and, if necessary, adjusted. The new crops should fit in current crop rotation systems. All these aspects together determine the economic feasibility of crop production. This stage is focused on identification of possible agronomic constraints and the definition of criteria for actual breeding. Breeding most often serves a dual purpose: strengthening of desired, and elimination of undesired features. This requires sufficient genetic variation, effective screening methods, and adequate

Table 1.2. The '4-ex' model for development of a new oilseed crop and product(s), with an approximate timetable.

Development stages	Time scale (years)							
	1	3	5	7	9	11	13	15
	-2	-4	-6	-8	-10	-12	-14	≥16
<b>1. Exploration</b>								
a: <sup>1)</sup> Germplasm collection/evaluation	----- <sup>2)</sup>							
i: Chemical and physical properties	-----							
<b>2. Examination</b>								
a: Agronomy and breeding	----->							
i: Application research	----->							
<b>3. Expansion</b>								
a: Crop production on farmers level	----->							
i: Pilot plant level production	----->							
<b>4. Exploitation</b>								
a: Large scale crop production	----->							
i: Commercialisation new product(s)	----->							

<sup>1)</sup> a,i = agriculture and industry, respectively

<sup>2)</sup> -----, - - - - = intensive and less intensive, respectively.

selection and recombination methods, which take into account the mode of reproduction of the crop (asexual vs. sexual, predominantly cross- or selffertilising), nature of the traits (qualitative or quantitative), heritability of the traits and genotype by environment interactions. If the available genetic variation for important features is limited, increase of variation by means of mutation or biotechnological techniques could be considered. Further crop improvement is a continuous and cyclic process of selection and recombination, which is in general very time consuming. The time required for transformation of a wild population into a more or less domesticated crop, even with the use of modern knowledge and techniques, should be expressed in terms of decades rather than years.

As soon as sufficient and representative quantities of seeds are available, effort must be put in oil recovery studies, comprehensive chemical analyses and physical property measurements. Processing procedures must be examined or developed, and possible uses identified.

### Stage 3. Expansion

In the expansion stage, the results of the previous stages, i.e. improved plant material, optimal techniques for cultivation, harvest and processing, and expected economics, will be used to lift the production and processing from a pilot-scale level to a (pre-)commercial level. Several farmers will test the performance of the new crop in current crop rotation systems, using 'standard' equipment. These trials should generate sufficient seed for a proper analysis of the processing costs and technical feasibility, in laboratories and pilot plants. When the new raw material simply replaces existing chemical components, properties of the products based on the new raw material should be tested. In case of components which are new to the industry, new end-uses need to be developed and subsequently tested. In this stage, many groups will become involved such as farmers, seed

producers, equipment suppliers, agrochemical manufacturers, regulatory agencies, initial and final processors, marketeers and final users.

#### *Stage 4. Exploration*

Commercialisation of a product cannot be successful without a market to absorb it. Products based on substitution of existing raw materials should be less or at the most, equally expensive, preferably be of higher quality, or have other beneficial properties (e.g. more environmentally friendly). New products based on industrially new raw materials need a sound reason for production. Commercial production requires that all stages of the production chain, from seed production via processing to production of the end-product, are or can be made economically feasible.

As can be seen from the previous, the development of a new crop involves many different disciplines and fields of expertise, as well as many types of organisations. In most 'western' countries, stages 1 and 2 and the first part of stage 3 are carried out within governmental and/or academic research centres. As crop development progresses, gradually the responsibility is passed over to private industry (including breeding companies and growers). The whole chain from development of new crops to the production of new products includes a large number of links. Each of these must be strong to ensure success, a single weak link in the chain may already result in failure.

### **History of new oil crops research in the Netherlands**

Arable farming in the Netherlands can be characterised as small-scale and highly intensive. For decades, potatoes, sugar beets and grain crops have made up about 80% of the total arable farming area. Sugar beets and potatoes are generally profitable crops, but susceptible to a number of pests and diseases, several of which are soilborne. Cultivation of these crops requires application of large volumes of agrochemicals, particularly in the current narrow rotation systems. Within such a system, the use of a monocotyledon crop (as winter wheat) to control disease-related problems is a prerequisite (Mulder *et al.* 1992, Van Soest *et al.* 1991). The Commission of European Communities (EC) has for considerable time financially supported the cultivation of small grains, but during recent years this support has decreased steadily, reducing the profitability of the crop. Furthermore, improved cultivars and cultivation practices in general resulted in substantially higher yields, leading to an overproduction of the major food and forage crops in Western Europe (Murphy 1994). In the meantime, Dutch governmental regulations have become quite restrictive on the use of agrochemicals, putting an extra pressure on the cultivation of crops as sugar beets and potatoes. One of the potential solutions to these problems could be to widen the crop rotation by introducing new crops with non-food/forage applications.

In the Netherlands, until 1986 only a few scientists showed interest in new industrial crops. Some new crops were introduced with reasonable success, albeit for a short period of time and on a small scale (Mulder *et al.* 1992).

**National Oilseeds Programme (NOP)**

Duration: four years (1990-1994)

Funding: Dutch Ministry of Agriculture, Nature Management and Fisheries

Participants: nine Dutch research institutes

Oilseed crops: *Calendula officinalis*, *Crambe abyssinica*, *Dimorphotheca pluvialis*, *Euphorbia lagascae*, *Lesquerella* spp., *Limnanthes* spp., *Lunaria annua*, *Osteospermum ecklonis*

Industry was represented in the advisory board.

**Vegetable Oils for Innovation in Chemical Industries (VOICI)**

Duration: four years (1991-1995)

Funding: 50% Commission of the European Communities (EC-ECLAIR programme)

Participants: four research institutes, two agricultural co-operatives and six industries, from four EC countries.

Oilseed crops: *Crambe abyssinica*, *Dimorphotheca pluvialis*, *Limnanthes* spp.

Industry participated in application research

**Vegetable Oils for Specific Fatty Acids (VOSFA)**

Duration: four years (1994-1998)

Funding: 50% Commission of the European Communities (EC-AIR programme)

Participants: five research institutes, two agricultural co-operatives and four industries, from five EC countries.

Oilseed crops: *Calendula officinalis*, *Dimorphotheca pluvialis*, *Euphorbia lagascae*, *Lesquerella* spp., *Lunaria annua*.

Industry participated in application research

Figure 1.2. Research programmes on potential oilseed crops in the Netherlands from 1990-1998 (adapted from Van Soest 1994).

In 1986, the Centre for Genetic Resources of the Netherlands (CGN, now part of Plant Research International) started collecting, introducing and evaluating samples of potential crops producing oils, fibres and carbohydrates, and in 1987 an initially small research programme on new crops was started. In this programme five research centres (for research on genetic variation, breeding, crop physiology, cultivation practices and processing) participated. The Ministry of Agriculture, Nature Management and Fisheries (LNV) of the Dutch government recognised the increasing problems of the arable farmers and funds were made available to extend this programme to a number of multidisciplinary programmes focusing on a specific product category and/or crop. In 1990, the 'National Oil Programme' (NOP) for fatty oils from potential oilseed crops was started, initially funded by LNV for a period of four years. The NOP focused on the agricultural development, breeding and agronomy, as well as the applicability of the oils and by-products (seed meal) of eight potential crops. Also, market studies and chemical/analytical research on the oils and main fatty acids were conducted. In the NOP, nine agricultural research centres participated, and several industrial companies were represented on the advisory board. In 1991, another four year project on oilseeds was initiated in the framework of the ECLAIR (European Collaborative Linkage of Agriculture and Industry

through Research) programme of the Commission of the European Communities. This project was called VOICI (Vegetable Oils for Innovation in Chemical Industries), and was a co-operation between agricultural research institutes, industrial partners and agricultural co-operatives from four EC countries. The activities within VOICI were directed towards three promising oilseed crops, of which genetic improvement, husbandry practices, oil recovery, processing technology and end-use research were studied. It also included a small demonstration component to scale up production of seeds and oils to the level of agricultural and industrial practice. Both NOP and VOICI were co-ordinated from the Centre of Plant Breeding and Reproduction Research (CPRO-DLO, now part of Plant Research International) and worked complementary. In 1994, when both NOP and VOICI came to an end, research on oil crops continued in a second four year project co-financed by the EC: Vegetable Oils for Specific Fatty Acids (VOSFA). An overview of the three projects is presented in Figure 1.2 (Van Soest and Mulder 1993, Van Soest 1994). The work presented in this thesis was carried out in the framework of both VOICI and VOSFA.

### ***Dimorphotheca pluvialis***

One of the species which received considerable attention within the oilseeds research programmes in the Netherlands is *Dimorphotheca pluvialis* (L.) Moench (Figure 1.3). Both the industrial and agricultural potential of this species looked promising. Seed oil from *D. pluvialis* contains large amounts (ca 60-65%) of dimorphecolic acid ( $\Delta^9$ -hydroxy,10t,12t-octadecadienoic acid; see Table 1.1). The highly reactive conjugated hydroxydiene structure of this fatty acid suggests that (after chemical modification) it is a versatile raw material in specialty applications, e.g. flavours and fragrances, pharmaceuticals, coatings, lubricants, plastic foams and nylons (Derksen *et al.* 1993, Knowles *et al.* 1965, Muuse *et al.* 1992).

The genus *Dimorphotheca* Vaill. ex Moench comprises eight species (Norlindh 1943 and 1980), of which *D. pluvialis* and *D. sinuata* DC. are the most important. Both species are diploid ( $2n=2x=18$ ), and originate from the winter rainfall region of the south-west Cape and Namaqualand (South Africa), where they replace one another geographically from south to north; *D. pluvialis* occurs mainly in the Cape region, while *D. sinuata* has its main centre of distribution in the Namaqua region (Norlindh 1946 and 1977). The species are difficult to distinguish except for flower colour: *D. pluvialis* has white flowers, while those of *D. sinuata* usually are bright orange. Where both species grow together, natural hybrids are frequently found. The flower colour of the hybrids varies from creamy white, pale buff, salmon, pale yellow to bright yellow. Both species and the hybrids are very decorative, and have already attracted the attention of horticulturists long ago. Drawings and descriptions of specimens of *D. pluvialis* growing in the botanical gardens in Leiden, produced by several botanists at the end of the 17th century, show that the species was already cultivated in the Netherlands as a garden





Figure 1.3. *Dimorphotheca pluvialis*: branch with flower and seed heads, unwinged seed (left), winged seed (right). (Drawing by Dorothee Becu, 2000)

ornamental three centuries ago (Norlindh 1943).

*D. pluvialis* is an annual herb, with individual plants showing considerable variation in plant architecture. Usually, one main stem is formed, which branches particularly at the base. When widely spaced, plants show strongly ramified basal branches. The appearance of the plants ranges from rather compact, with relatively short, firm branches to very open, with procumbent to almost prostrate basal branches. Depending on the architecture, plant height is 10-60 cm and plant diameter 30-100 cm. Individuals growing in very dry or

innutritious habitats show reduced branching and ramification, and leaves which are relatively small, hairy and hardly indented or lobed. In more moist and nutritious conditions, shape, size and pubescence of the leaves show much more variation, but most often particularly the basal leaves are substantially larger and only sparsely haired. These plants also tend to have more branches which are more ramified. Although environmental conditions affect growth habit, the variation for plant architecture in *D. pluvialis* is undoubtedly (partially) due to genetic differences too (Norlindh 1943).

The flowers are situated at the distal ends of the branches, and are very conspicuous. As is typical for members of the family of the *Compositae* (*Asteraceae*), the flower is in fact a flower head or capitulum, bearing two types of florets. The disk florets have a tubular, 5-lobed corolla which, depending on genotype, can vary in colour from bright yellow to dark purple. The inner disk florets of a capitulum often have appendages on the corolla lobes, the size of which usually decreases towards the outer disk florets. The filaments of the five anthers have grown together, forming a tube. The style has two short lobes (1 mm), which diverge after the style has grown through the anther tube. The pollen is orange or yellow, and rather sticky. The ray florets have one large petal which is white except near the base, where they have a dark blueish or reddish purple zone, which can vary in width between genotypes. Sometimes it is very narrow and scarcely noticeable, but particularly in the types cultivated as ornamental the coloured zone can be very wide, up to 1 cm, giving the appearance of a large 'ring' in the flower. This 'ring' does not show through at the back side of the petal, which itself shows considerable variation in colour: streaks of blueish-violet-purple, often with an interspersation of copper, and less often brown. Also genotypes without these pigments are found; in this case the petals of the ray florets are completely white on the upper side and white and yellow beneath, and the disk florets are yellow. The anthers of the ray flowers are rudimentary. The style is deeply cleft at the top, forming two large, diverging lobes (Hoffmann 1894, Norlindh 1943 and 1977). The number of ray florets per capitulum ranges between 11 and 21, with peaks at 13 and 16. Correns (1906a) found that this variation is primarily due to external influences, for instance in less nutritious environments the heads are smaller and have fewer ray florets. The open flower heads are as a rule 4-6 cm in diameter. However, under poor environmental conditions the size of flower heads may be as small as 2 cm across, while under luxuriant conditions it may even reach 9 cm.

*D. pluvialis* is described as a predominantly outcrossing species. The actinomorphic, radiate shape of the capitulum attracts a wide range of insect visitors, mainly species from the *Coleoptera*, *Diptera*, *Lepidoptera* and particularly the *Hymenoptera*, most of which are known to be able to contribute to pollination (Leppik 1977, Proctor and Yeo 1973). Self-pollination (and -fertilisation) however, cannot be ruled out. It is possible that pollen is transported within the same floret, capitulum, or between capitula of the same plant, all of which effectively is self-pollination. No self-incompatibility has been reported in this species.

The disk florets are hermaphrodite and the ray florets are only female fertile. Both types of florets produce distinctly different achenes (dry, one-

seeded fruits with the seed distinct from the pericarp), often simply referred to as 'seeds'. To this feature, *Dimorphotheca* owes its generic name: *di* => two, *morphe* => shape, *theca* => seed/capsule. The achenes produced by the ray florets are more or less rod-shaped, angular and frequently tuberculate, while those of the disk florets are smooth, flattened, and variously cordate to oval in outline with winged margins (Barclay and Earle 1965, Norlindh 1943). Thousand 'seed' weight of the unwinged achenes is usually somewhat higher than of the winged achenes, approx. 2.8 and 2.5 g, respectively (Correns 1906a, Rutgers 1991). Both types of achenes have a similar oil content and fatty acid composition (Earle *et al.* 1964, Meier zu Beerentrup and Röbbelen 1987). The oil is situated in the embryo, as is common in most annual oilseed crops, and serves as a source of energy for seed germination (Stymne 1992). Oil content ranges from 15-25% (Barclay and Earle 1965, Derksen *et al.* 1993, Rutgers 1991). Besides 60-65% dimorphecolic acid, the oil contains 10-20% oleic acid, 10-14% linoleic acid, 2-3% palmitic acid, 1-2% stearic acid, and small amounts (<1.5%) of other fatty acids (Binder *et al.* 1964, Muuse *et al.* 1992 and 1994). Disk achenes germinate somewhat better and more quickly than ray achenes. This is probably caused by the different structure of the pericarp, since the germination rates were almost equalised when the pericarp was removed from the seeds (Correns 1906b, Becker 1913).

*D. pluvialis* has a variety of common names, some referring to its centre of distribution, and many to the colour and appearance of its flower: 'African Daisy', 'Cape Daisy', 'Rain Daisy', 'Ox-eye Daisy', 'Witbotterblom', 'Wit sôe', 'Witmargriet', 'Bietou' or 'Reeënblommetjie' (Adamson and Salter 1950, Bond and Goldblatt 1984, Eliovson 1973, Le Roux and Schelpe 1981, Phillips 1951, Smith 1966). The species' epithet '*pluvialis*' refers to the plant's tendency to close its flowers when dark and rainy weather is at hand. It was thought for some time that the movement of the flowers during the day actually predicted forthcoming rainfall, hence the plant was called 'weather prophet'. Royer (1868) noticed however, that closing of the flowers only occurred when rain was accompanied by a decrease of temperature. Later, Heinricher (1923) discovered that the movement of the flowers was not induced by air humidity nor radiation, but solely by temperature: flowers do not open below 14-17 °C. The closing of open flowers could be induced in the brightest daylight by cooled air, the opening and closing of the flower heads could take place in constant darkness by changes of temperature, and closed flower heads could always be forced to open by rises in temperature, irrespective of their being in light or darkness.

### **Agronomy and breeding of *Dimorphotheca pluvialis***

Although species of the genus *Dimorphotheca* have been known in the Netherlands as a garden ornamentals for several centuries, the use of *D. pluvialis* for the production of seed oil is completely new. No breeding with regard to its use as an arable seed crop has been reported, and as such the species should be considered as undomesticated. Plant populations selected

for ornamental purposes generally have little value as arable crops, since both types of crops have to meet different, sometimes even opposite, criteria. For use as a garden ornamental, variation in plant architecture and flower characteristics as well as a long flowering period are welcomed, while seed set, seed retention, and content and quality of seed oil are irrelevant features. It is obvious that for commercial seed oil production the latter characteristics are highly relevant, in combination with a uniform plant architecture, and relatively short periods for flowering and seed ripening.

In an evaluation trial at five locations in the USA, Knowles *et al.* (1965) experienced difficulty in growing *Dimorphotheca*, primarily because of insects, nematodes and fungal diseases (*Alternaria* spp., *Botrytis* spp., and *Fusarium* spp.). These authors also found that seed retention was poor, and gave no reliable data on seed yield. Analyses showed that the seed hull made up about 63% of the total seed weight, the average oil content of dehulled seed was 27%. Willingham and White (1973) also reported the susceptibility of *Dimorphotheca* to several diseases in the USA: *Plasmopara halstedii* (Farl.) Berl. & de Toni, *Cephalosporium* spp., *Fusarium* spp., *Pythium ultimum* Trow., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Verticillium* spp., *Alternaria* spp., *Botrytis cinerea* Pers., *Puccinia flaveriae* Jacks. and *Rhizoctonia solani* Kühn. The research of the latter authors focused mainly on phenological and morphological characteristics of *Dimorphotheca* and *Osteospermum* spp. at very low plant densities, and no reliable data on yield were available. In the 1980s, an extensive field evaluation of seven promising oilseed crops, including *D. pluvialis*, was performed in Germany (Meier zu Beerentrop 1986, Meier zu Beerentrop and Röbbelen 1987). These authors reported seed yields ranging from ca 800 kg/ha (combine harvested after desiccation) to ca 1600 kg/ha (harvested by hand at maturity), and observed severe seed shattering. Oil content of the (hulled) seeds was low, 15.8%, and dimorphecolic acid content of the oil 62%. Vegetative growth and plant development were satisfying and as far diseases are concerned only an attack of powdery mildew (not specified) after flowering was reported.

In the Netherlands, between 1988 and 1990 several small scale evaluation trials were conducted, of which the reported seed yields were rather erratic. Potential seed production of the used populations was about 1800 kg/ha with an average oil content of 21%, but seed losses up to 75% of the total yield occurred due to seed shattering. The optimal time of harvest was difficult to assess because of the long, unsynchronised period of flowering and seed ripening (Rutgers 1991, Wingelaar 1990).

### Scope of this thesis

The research described in this thesis was carried out at CPRO-DLO between 1991 and 1997, in the framework of two successive multidisciplinary projects (see Figure 1.2), in which CPRO-DLO was responsible for the genetic evaluation and breeding research of *D. pluvialis*. As only few scientific papers on this species had been published at that time, the options for

research subjects were numerous. As the objectives of the project were rather pragmatic, initially it was decided to focus on the major constraints limiting production of the oil, namely seed set, seed retention, flowering synchronisation and oil content. Preliminary investigations on seed retention, however, revealed little variation for this character, and the observed variation seemed to be primarily caused by differences in environmental conditions, masking possible genetic variation (unpublished data). Taking into account the relatively short duration of the project, it was considered more effective not to pursue the research on seed retention, but to further concentrate on the synchronisation of flowering instead. Emphasis was laid on heritability studies of flowering characteristics as well as oil content, in order to facilitate the development of efficient breeding strategies.

To the author's knowledge, the influence of pollinating insects on seed set and other relevant yield components has not previously been described. In the available literature, *D. pluvialis* is considered an allogamous species, and insects are the most likely vectors for pollen transfer (Leppik 1977, Proctor and Yeo 1973). Therefore, *D. pluvialis* was grown under conditions of both presence and absence of insects, and the effects on yield components were studied. The results of this experiment are presented in Chapter 2. Next to seed yield, oil content of the seeds is the second important factor determining oil yield. High and stable oil yields of good quality are essential to provide industry with a constant supply. As yet, oil yield of *D. pluvialis* is too low for an economically feasible production, and oil content of the seeds needs to be increased. In Chapter 3, realised response to mass selection and estimation of heritability for increased oil content in three populations are described. Estimations of heritability for oil content were also determined from parent-offspring regression and from half-sib family variance components, the results of which are described in Chapter 4. In this chapter, not only oil content was investigated, but also heritability estimates of onset of flowering are presented. As explained in Chapter 5, synchronisation of flowering is not achieved when only the onset of flowering between plants is synchronised. The synchronisation of flowering within plants (flowering period) also needs to be optimised. In this chapter, a model describing the flowering of plants is introduced, and heritabilities of flowering traits are estimated. In Chapter 6, selection for onset of flowering was combined with selection for different types of plant architecture. Both the response to selection and the effect of selection for these traits on seed yield are discussed. A state of the art on *D. pluvialis* research with a crop perspective and suggestions for further research from an agronomical and breeder's point of view, is presented in Chapter 7.

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## Chapter 2

### **The influence of insect pollination on yield components in *Dimorphotheca pluvialis***

#### **Summary**

*Dimorphotheca pluvialis* (L.) Moench is currently investigated for crop potential as a source of dimorphecolic acid. Seed yield is rather low and erratic, possibly partially as a result of an inferior seed set. The effect of the absence of insects on yield components was studied using three populations of *D. pluvialis*. Insect-free environments were created by covering small field plots with cages. The cages had no effect on plant and flowering development. In the absence of insects the populations flowered longer and produced fewer seeds with a lower oil content. The combined effect of low seed yield and low oil content in insect-free environments resulted in an 80% reduction of oil yield compared to insect-visited environments. Clearly, in *Dimorphotheca pluvialis* insects are very important with regard to seed set and oil yield.

#### **Introduction**

The introduction of new oil crops for the production of vegetable oils for industrial uses is currently under intensive investigation. The diversification in agriculture by introducing novel crops is expected to lead to a reduction in agricultural surpluses, and a broadening of the existing, narrow crop rotation schedules (Van Soest and Mulder 1993, Van Soest 1990, Van Soest 1994). Furthermore, oilseed crops can provide industry with new, unusual fatty acids which can not only replace certain petrochemicals with renewable resources, but also expand the existing range of oleochemical base materials (Derksen *et al.* 1993, Muuse *et al.* 1992, Princen 1982). In the early sixties, a wide-scale programme for the development of new industrial crops from wild species was started by the U.S. Department of Agriculture (Barclay and Earle 1965, Earle *et al.* 1964, Jones and Earle 1966, Princen 1983). In Europe, particularly in the past decade several new oilseed crops with a unique fatty acid composition have been tested for feasibility of oil production for industrial uses (Meier zu Beerentrup and Röbbelen 1987, Van Soest and Mulder 1993). At CPRO-DLO (now part of Plant Research International), since 1986 more than 40 potential oilseed crops were evaluated for crop adaptation and important agricultural characteristics. After considering the industrial interest and agricultural potential, *Dimorphotheca pluvialis* (L.) Moench was one of the few species selected for further breeding research (Van Soest and Mulder 1993, Van Soest 1994).

*D. pluvialis* is a herbaceous annual, native to southern Africa, particularly the Cape region (Norlindh, 1977). The species is well adapted to the maritime climate of north-west Europe, and fits in an agricultural rotation system with annual crops. Although some species of *Dimorphotheca* are known as garden ornamentals, *D. pluvialis* is considered an undomesticated species for



seed production. Plant populations in general have a long, unsynchronized flowering period and show poor seed retention (Hof 1996). As is common in the *Asteraceae* (*Compositae*), the flower is in fact a capitulum with two distinct types of florets. In the genus *Dimorphotheca* the disc florets are hermaphrodite, and the ray florets are female fertile, but male sterile. The ray florets have one large white petal which is often coloured purple at the base, giving the appearance of a 'ring' in the inflorescence (Barclay and Earle 1965). Both types of florets produce distinctly different types of seeds (achenes). Seeds produced by the ray florets are small, rod-shaped, while those of the disc florets are flattened and have two large wings. The seeds contain ca 21% oil, with 60-65% dimorphecolic acid ( $\Delta^9$ -hydroxy,10t,12t-octadecadienoic acid), a very specific fatty acid with a chain length of 18 carbon atoms, with two conjugated double bonds and a hydroxy group. The structure suggests that this fatty acid is chemically very reactive and hence suitable for a wide range of industrial products such as surface coatings, surfactants, plastic foams, flavours and fragrances (Muuse *et al.* 1992, Knowles *et al.* 1965).

At present, oil yields of *D. pluvialis* are too erratic to provide industry with a constant supply. Oil production, and thus seed yield needs to be stabilised and preferably increased. An important component of seed yield is seed set. Seed production might be limited by inadequate pollination and subsequent fertilisation. In the present paper results of investigations on the effect of pollinating insects on seed set and other relevant yield components of *D. pluvialis* are presented.

### Material and methods

The plant material used consisted of three populations of *Dimorphotheca pluvialis*, 879127, 879585 and 879731, originating from botanical gardens in Berlin (Germany), Dniepro Petrowsk (Ukraine) and Århus (Denmark), respectively. All three populations have been used in field evaluation trials at CPRO-DLO between 1990 and 1993 (Mulder 1995). Before using these populations for the experiments described here, the seed material was subjected to seed germination tests and sowing rates were adjusted accordingly.

The influence of insects on seed set in *D. pluvialis* was studied by comparing insect-free with insect-visited environments. An insect-free environment was created by covering small field plots with cages. The cages allowed wind pollination, but excluded insects. Insect-visited environments were created by small field plots without cages, as well as small field plots covered by a cage which was left open at the north side, allowing insects to visit. The open-cage environment was included to determine possible effects of shading by the cage on plant and flowering development.

The experimental layout was a completely randomised block design with three replicates, three pollination environments, and the three populations of *D. pluvialis*; within blocks all nine possible combinations of pollination

environments and populations were randomly allocated to a plot. Plot size was 3 x 3 m and cage size was 3 x 3 x 2 m. Plots were separated 6 m in east-west direction, and 3 m in north-south direction to minimise shading effects of the cages between plots.

Plots were sown on April 22, 1994, on loamy clay soil near Lelystad, the Netherlands. Cages were placed three weeks before onset of flowering. Plant density of the plots was determined by counting plants on a 0.5 m<sup>2</sup> subplot. Flowering development was scored by counting the open flowers of the same subplot five times, with weekly intervals, starting ten weeks after sowing. One week before harvest 20 seed heads were collected randomly per plot, of which seed set was determined by counting the developed seeds. Plots were harvested 15 weeks after sowing, by mechanically cutting the whole crop. Shattered seed was collected by vacuuming the soil surface with a household vacuum cleaner and subsequently sieved to remove soil particles. The plant material was dried, threshed and separated from debris afterwards. During the seed cleaning process the two seed types were separated. Per plot yield of winged and unwinged seeds (including shattered seeds), total seed yield and thousand seed weight of winged and unwinged seeds were determined. Oil content was determined by near-infrared spectrometry (Bran & L bke InfraAlyzer 500), using the winged seeds. Oil content of unwinged seeds was not determined; earlier experiments showed that both types of seeds have a similar oil content, and winged seeds constitute the larger part of the total seed yield (F. Mulder, CPRO-DLO Wageningen, pers. comm.). Oil yield was calculated from total seed yield and oil content.

Analysis of variance was performed on all characteristics. To examine the possible effect of plant density on flowering development, in the analyses of the numbers of flowers a polynomial with plant density as explanatory variate was fitted within the factor population. By doing so, the sum of squares for the factor population is partitioned into an amount that can be explained by a linear relationship between the number of flowers and plant density, and a residual. The interaction between population and treatment is similarly partitioned. For all computations, 'Genstat 5' software (Genstat 5 Committee 1989) was used.

## Results and discussion

### *General*

Seedlings emerged two to three weeks after sowing. The first flowers opened 9.5 weeks after sowing. During the growing season no severe attacks of pests or diseases were observed. At harvest about 19% of the seed yield of the insect-visited plots was collected from the soil. These shattered seeds were included in the seed yield to obtain a more accurate estimate of the seed production. On the insect-free plots no obvious seed shattering had occurred. Means and results of analyses of variance are presented in Table 2.1.

Table 2.1. Plant density, flowering, seed set, oil and seed yield characteristics in insect-free and insect-visited environments, of three populations of *Dimorphotheca pluvialis*.

Characteristic	Pollination environment			F-test <sup>2)</sup>	LSD <sup>2)</sup>
	without cage	open cage	closed cage		
Number of plants (0.5 m <sup>2</sup> )	50.8	55.3	46.7	n.s.	-
Number of open flowers (0.5 m <sup>2</sup> ):					
10 weeks after sowing	2.8	1.9	1.4	n.s.	-
11 weeks after sowing	81.2	67.6	63.8	n.s.	-
12 weeks after sowing	297.8	284.2	359.4	*	50.2
13 weeks after sowing	165.8	143.2	305.2	***	32.5
14 weeks after sowing	15.0	8.0	141.0	_ <sup>3)</sup>	_ <sup>3)</sup>
Seed set unwinged seeds <sup>1)</sup>	14.4	15.4	2.4	***	1.1
Seed set winged seeds <sup>1)</sup>	41.3	41.2	5.1	***	5.0
Yield of unwinged seeds (g/m <sup>2</sup> )	40.1	30.6	13.9	***	3.7
Yield of winged seeds (g/m <sup>2</sup> )	85.8	52.4	13.5	***	6.8
Total seed yield (g/m <sup>2</sup> )	125.9	83.0	27.4	***	9.2
Thousand seed weight unwinged (g)	2.90	3.03	4.46	***	0.16
Thousand seed weight winged (g)	2.14	2.20	2.46	***	0.08
Oil content (% , ca 8 % moisture)	21.6	22.1	17.8	***	0.8
Oil yield (g/m <sup>2</sup> )	27.1	18.3	4.9	***	2.0

Characteristic	Population number			F-test <sup>2)</sup>	LSD <sup>2)</sup>
	879127	879731	879585		
Number of plants (0.5 m <sup>2</sup> )	40.7	45.3	66.8	***	7.9
Number of open flowers (0.5 m <sup>2</sup> ):					
10 weeks after sowing	2.3	1.3	4.4	**	1.6
11 weeks after sowing	56.6	59.2	96.6	***	19.5
12 weeks after sowing	281.8	269.2	390.6	***	50.2
13 weeks after sowing	242.0	214.0	148.2	***	32.5
14 weeks after sowing	45.6	52.2	66.2	_ <sup>3)</sup>	_ <sup>3)</sup>
Seed set unwinged seeds <sup>1)</sup>	10.7	10.3	11.1	n.s.	-
Seed set winged seeds <sup>1)</sup>	29.0	30.2	28.4	n.s.	-
Yield of unwinged seeds (g/m <sup>2</sup> )	26.8	28.3	29.5	n.s.	-
Yield of winged seeds (g/m <sup>2</sup> )	43.9	57.4	50.4	**	6.8
Total seed yield (g/m <sup>2</sup> )	70.7	85.7	79.8	*	9.2
Thousand seed weight unwinged (g)	3.39	3.50	3.48	n.s.	-
Thousand seed weight winged (g)	2.26	2.28	2.25	n.s.	-
Oil content (% , ca 8 % moisture)	21.3	19.5	20.7	***	0.8
Oil yield (g/m <sup>2</sup> )	15.6	17.4	17.2	n.s.	-

<sup>1)</sup> Number of unwinged and winged seeds per seed head, mean of 20 seed heads.

<sup>2)</sup> Level of significance of F-test: \*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$ ; n.s. = not significant; LSD<sub>(0.05)</sub> = least significant difference at 5% level.

<sup>3)</sup> No main effects presented because of population by pollination environment interaction.

### *Plant density and flowering development*

Plant establishment was satisfactory, the aimed plant density of 80-100 plants/m<sup>2</sup> was achieved on almost all plots. The pollination environments had no influence on plant density, since the cages were placed after plant density was scored. Population 879585 showed a significantly higher plant density than populations 879127 and 879731, despite seed germination tests and adjusted sowing rates. Possibly the tests underestimated the germination capacity of this population, or the germination conditions were favourable for this population in particular.

In the course of flowering, the influence of the different pollination environments on flowering development became clearly visible. Twelve weeks after sowing the insect-free environment showed a few more flowers than the insect-visited environments ( $P \leq 0.01$ ). One week later this difference was much more pronounced ( $P \leq 0.001$ ). While at this stage in the insect-visited environments the number of open flowers decreased, flowering continued in the insect-free environment. No significant differences were found between plots without cages and plots with open cages, indicating that the cage had no adverse effect on flowering development.

Populations 879127 and 879731 showed a similar pattern in flowering development; both populations started flowering at the same time, and produced almost equal numbers of flowers in the course of time. Population 879585 started flowering a little earlier, and the number of flowers at peak bloom was higher. Because plant density is known to affect plant and flowering development (Kreuzer *et al.* 1996), analyses of variance on number of flowers were carried out with plant density as explaining variable within population. In the earlier stages of flowering (10 and 11 weeks after sowing) only the factor population was significant. At 12 weeks after sowing, both population and pollination environment became significant, but no interactions were found. At 14 weeks after sowing, a significant population by pollination environment interaction was found ( $P = 0.006$ ). This interaction was primarily due to very high numbers of flowers produced by population 879585 in the closed cages. Within the population and population by pollination environment interaction, the linear component of the polynomial was highly significant ( $P = 0.002$  at 10 weeks after sowing, and  $P \leq 0.001$  at 11, 12, 13 and 14 weeks after sowing), and the residuals of the polynomial were not significant. These results indicate a linear relationship between plant density and number of open flowers, plots with higher plant densities produced less flowers. However, the observed differences in plant density were confounded with the populations. Regression analysis of number of flowers on plant density after elimination of variation due to pollination environment and population, revealed no significant relationship which would have supported the hypothesis that plant density was the cause of the observed variation. From these data, it could not be determined whether the differences between the populations for number of open flowers could be attributed to differences in plant density or genuine differences between populations.

### Seed set

In the insect-free environment seed set was much lower than in insect-visited environments ( $P \leq 0.001$ ). On average 15 unwinged and 41 winged seeds per seed head were found on insect-visited plots, against 2 unwinged and 5 winged seeds per seed head on insect-free plots. There were no significant differences between plots without cages and plots with open cages, so it must be assumed that the severely reduced seed set in the closed cage environment was due to the absence of insect pollinators. Since in the absence of insects some seeds were formed, it is likely that self pollination and/or vectors other than insects are involved in seed set. However, the degree of decrease in seed set in the insect-free environment indicates that insects are by far the most important vector for pollen transfer.

This experiment did not give an answer to which insects may have been important for pollination. Observations on other *D. pluvialis* fields revealed that flowers were frequently visited by different butterflies as painted ladies (*Cynthia cardui* (L.)) and geometrid moths (*Geometridae*), buff-tailed bumble bees (*Bombus terrestris* (L.)), honey bees (*Apis mellifera* L.), drone flies (*Eristalis* spp.), hover flies (*Syrphidae*) and blow flies (*Calliphoridae*). All listed insects are known to be able to contribute to pollination (Y. Jongema, Agric. Univ. Wageningen, the Netherlands, pers. comm.).

Seed set was not significantly different between populations.

### Seed yield

Between the pollination environments distinct differences were found. Thousand seed weight of both types of seed was higher from the insect-free environment than from insect-visited environments ( $P \leq 0.001$ ). Although the number of flowers in the insect-free environment was also substantially higher than in the insect-visited environments, these two factors were not able to compensate the low seed set, resulting in a much lower seed yield for this environment ( $P \leq 0.001$ ). The total seed yield of plots without cages was about five times higher than of plots with closed cages.

Although no differences in plant density, number of flowers, seed set and thousand seed weight were observed between cageless plots and open cage plots, seed yield of both types of seed was significantly lower in the latter treatment. After storage, in the seed bags of this treatment numerous moths were found. Already during the experiment many moths were observed at the ceiling of the open cages. It is likely that juvenile stages of the moths have been harvested with the seed and pupated during storage. Caterpillars of moths may have destroyed flowers or developing seeds prior to harvest, though this was not very conspicuous. No other explanation for the yield reduction was found.

Between populations, the differences were less pronounced. No significant differences were found for yield of unwinged seeds, but yield of winged seeds was somewhat lower for population 879127 as compared to the other two populations ( $P \leq 0.01$ ). Consequently, this resulted in a slightly lower total seed yield for that population ( $P \leq 0.05$ ). No significant differences were found for thousand seed weight of either winged or unwinged seeds.

### Oil content and oil yield

The difference in oil content between the pollination environments was obvious ( $P \leq 0.001$ ): seed obtained from the insect-free environment had a 4% lower oil content than seed obtained from insect-visited environments. The combined effect of low oil content and low seed yield of the closed cage plots, and the higher oil content and high seed yield of the cageless plots resulted in a 5.5 times higher oil yield for the latter environment. The lower oil yield of the open cage plots as compared to the cageless plots was entirely attributed to the lower seed yield of this pollination environment, since there was no difference in oil content between these environments.

Oil content was significantly different between populations ( $P \leq 0.001$ ), but the range was less than 2%. The average oil content in this experiment was 20.5%, which is in accordance with the findings of Mulder (pers. comm.).

### Conclusions

Exclusion of insects had a dramatic effect on the production of flowers and the duration of the flowering period. The number of flowers at peak bloom was higher and flowering continued longer in the insect-free environment. Seed set, seed yield and oil content were severely reduced in the absence of insects. This could not be compensated by the prolonged flowering period and higher thousand seed weight. The total seed and oil yield of plots without cages were respectively 4.5 and 5.5 times higher than of plots with closed cages, which clearly underlines the importance of insect pollination in *Dimorphotheca pluvialis*.

Flowering development was different for the populations, but since differences in plant density were confounded with populations it was not clear whether the observed differences were attributable to plant density effects or genuine differences between populations. Although no differences in seed set were found, the populations were slightly different with regard to yield of the winged seeds, and consequently total seed yield. The populations had an equal thousand seed weight for both seed types. The differences in oil content were relatively small, though statistically significant, but did not result in different oil yields of the populations.

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## Chapter 3

# Response to mass selection and estimation of heritability for oil content in *Dimorphotheca pluvialis*

### Summary

Three populations of *Dimorphotheca pluvialis* (L.) Moench were subjected to mass selection for increased oil content. After three cycles of selection, at a selection intensity of 10%, in all three populations a significant increase in oil content was observed. The average gain in oil content per selection cycle varied from 0.5% to 1.2%. Realised heritabilities ranged from 0.15 to 0.58. It is likely that the observed differences in response and heritability for oil content can be attributed to different genetic constitutions of the populations used. Though heritabilities are specific for populations, selection procedures and environmental circumstances, the observed values for the realised heritabilities indicate that a substantial part of the phenotypic variance is additive genetic variance. Therefore, continued (mass) selection for increased oil content will be effective in *D. pluvialis*.

### Introduction

In the past decades, the interest for vegetable oils as industrial raw materials has greatly increased (Lühs and Friedt 1994). Particularly oils with fatty acids containing functional groups are very attractive as substitutes for mineral oils used in the manufacturing of products like lubricants, surfactants, coatings and polymers, provided that the vegetable oils can be obtained at a constant supply and at moderate costs. Therefore, in recent years much effort has been made to domesticate wild plant species containing such oils (Princen and Rothfus 1984, Thompson *et al.* 1992, Van Soest 1990). The interest for industrial crops in the Netherlands has intensified since 1986, when the first of a series of projects on the potential of industrial crops was launched (Van Soest and Mulder 1993, Van Soest 1994). Around 40 oilseed crops have been evaluated for various agronomic characteristics as well as oil content and quality. After considering the industrial interest and agricultural potential, four crops were selected for further breeding research. One of these was *Dimorphotheca pluvialis* (L.) Moench, a white flowering, predominantly outcrossing, annual species of the *Compositae* (*Asteraceae*) family, native to South Africa and Angola. Flower heads of *D. pluvialis* are composed of female fertile, male sterile ray florets, and hermaphrodite disk florets. Seeds (achenes) developing from disk florets have flattened margins (wings), those produced by the ray florets are unwinged (Barclay and Earle 1965, Norlindh 1977). Both types of seed contain ca 21% oil with approximately 60-65% dimorphecolic acid ( $\Delta^9$ -hydroxy,10t,12t-octadecadienoic acid). This fatty acid is unique in the sense that it contains two conjugated double bonds relative to the  $\alpha$ -carbon of the hydroxy group. This feature provides dimorphecolic acid with a unique functionality and properties that are totally different from other known hydroxy fatty acids such as ricinoleic acid (from castor bean, *Ricinus*



*communis* (L.)) and lesquerolic acid (from *Lesquerella* sp.). This implies that a new spectrum of applications can be anticipated (Binder *et al.* 1964, Earle *et al.* 1964, Muuse *et al.* 1992). Market oriented studies showed that industries in the field of coatings, lubricants, polymers, flavours and fragrances have shown an interest in oil and/or its derivatives of *D. pluvialis* (Sengers and Koster 1995). Another application, the production of pheromones on the basis of oil of *D. pluvialis* is ready for commercialisation (J.T.P. Derksen, ATO-DLO Wageningen, pers. comm.).

For successful introduction of oil of *D. pluvialis* as raw material in chemical industry, crop production and oil content of the seed must be improved (Knowles *et al.* 1965, Meier zu Beerentrop and Röbbelen 1987). Also, procedures of processing and application of the oil should be optimised or developed (Muuse *et al.* 1992, Derksen *et al.* 1993). At CPRO-DLO (now part of Plant Research International), research on *D. pluvialis* has focused on genetic improvement of seed and oil production. Evaluation experiments revealed substantial variation for these characters, both between and within populations (Mulder *et al.* 1992, Hof 1996). In the present paper, results are reported on mass selection for increased oil content.

## Materials and methods

### *Plant material and selection procedures*

For the experiments, three populations of *D. pluvialis* were used, in which no prior artificial selection had taken place. The populations originated from botanical gardens in the Ukraine (population number 879585), Germany (population number 880588) and France (population number 880992). Seed of the three populations was kindly provided by the Centre for Genetic Resources in the Netherlands (CGN), now part of Plant Research International. The populations were chosen on account of their relatively high oil content, as observed in *Dimorphotheca* field evaluation trials at CPRO-DLO in 1990 and 1991 (F. Mulder, pers. comm.).

Mass selection for improved oil content was performed parallel in all three populations. Throughout the whole selection phase of the experiment the populations or derived selections were grown in spatial isolation. In the first year, per population 100 plants were grown and the seed of individual plants was screened for oil content. Equal portions of seed of the ten best performing plants were mixed and used for the next selection cycle. Between 1992 - 1994, three selection cycles were carried out following this procedure, except that in the 2nd and 3rd year the number of plants per population was increased to 125, whereas the proportion of plants selected was kept at 10%. For reasons of clarity in the text, populations 879585, 880588 and 880992 will hereafter be referred to as population A, B and C, respectively. The combination of a population and its derived selections will analogously be referred to as group A, group B and group C. The four selection generations, i.e. the population and three subsequent selections will be referred to as C<sub>0</sub>, C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub>, respectively.

In 1995, all populations and selections were sown together in one trial to assess the response to selection. The material was sown at two dates with a three weeks interval, in order to create different growing conditions within the same year and location. The trial field was divided into three sections, to each of which one group was allocated. Each section was divided into two subsections; one for each sowing date. Subsections consisted of three blocks (replicates), each with four rows (plots), to which the four selection generations were randomly allocated. Each row consisted of 14 plants (subplots). All plants were harvested individually and oil content determined accordingly. This experimental layout, with the allocation of the three groups to three sections, was chosen to minimise the error variance between selections of a group. The primary objective was the comparison of selections within a group, in order to determine the response to selection. The subdivision of the sections into two subsections for the two sowing dates was chosen for practical reasons only.

*Statistical procedures, determination of selection response and realised heritability*

Analysis of variance was carried out for each group separately, using Genstat (Genstat 5 Committee 1989). The effect of sowing date on oil content was determined by testing sowing date mean squares against block mean squares. The response to selection was calculated from the difference between two successive generation means:

$$R = \bar{X}_j - \bar{X}_{j-1} .$$

Since selection responses tend to fluctuate between generations, the average gain per selection cycle was estimated from the slope of a regression line fitted to the generation means. The realised heritability was estimated by the common technique of regression of cumulative response on cumulative selection differential (Falconer 1989, Hill 1972):

$$h^2 = \frac{R}{S} .$$

The selection differentials were estimated in the year of selection from:

$$s = \bar{X}_s - \bar{X} ,$$

where  $\bar{X}_s$  = mean of selected plants and  $\bar{X}$  = population mean before selection. By definition, the selection differential is highly dependent on the phenotypic variance of the population under selection ( $\sigma_p^2$ ). Since selection differential and response to selection of each cycle were estimated in different years, Mesken (1987) suggested to use a standardised response ( $R/\sigma_p^2$ ) and standardised selection differential ( $S/\sigma_p^2$ ) for estimation of the realised heritability (where  $\sigma_p^2$  denotes the phenotypic standard deviation of the respective populations). Assuming random mating and selection solely taking

place on the maternal parent, the realised heritabilities in this experiment were estimated by doubling the coefficient ( $b$ ) of the linear regression of cumulative standardised response on cumulative standardised selection differential:

$$h^2 = 2 \frac{R / \sigma_p}{S / \sigma_p} = 2b .$$

#### *Technical information*

All experiments were carried out at the CPRO-DLO experimental farm in Lelystad, the Netherlands. The soil type in this area is a silty clay with a very uniform soil structure. In the first year the trials were sown with a sowing machine at a row distance of 75 cm. Within rows plant distance was adjusted to 75 cm by removing surplus plants. In the following years trials were sown by hand at a plant spacing of 1 x 1 m. To avoid empty spaces, several seeds were sown per plant position and thinned to a single plant several weeks after emergence. Seed of individual plants was harvested by picking the seed heads when mature. Because of irregular ripening of seed heads of plants, harvest was carried out once a week until sufficient seed was collected for determination of oil content. After harvest, the seed was cleaned, partitioned into the two seed types and stored at 12 °C, 30% relative humidity, until further use.

Oil content of the winged seeds was determined by means of Near Infrared Reflectance Spectrometry (NIRS) using a Bran & Lübbe InfraAlyzer 500. Oil content of the unwinged seeds was not determined because both types of seed have a similar oil content and the winged seeds constitute the larger part of the seed yield (Earle *et al.* 1964, Meier zu Beerentrup and Röbbelen 1987). The used calibration line was based on 169 seed samples from several experiments conducted between 1990 and 1993, and suitable for the determination of the oil content of seed samples from different experiments (Groenendijk and Frankhuizen 1993). The standard error of these values was 1.3%.

## **Results and discussion**

### *Influence of sowing date*

To enable statistical comparison of the two sowing dates, in the analyses of variance mean squares of sowing dates were tested against block mean squares. It was assumed that there were no systematic differences between the two subsections because of the uniform soil structure. Therefore, only a slight underestimation of the error variance was expected, which would most likely not affect the conclusion. Using this procedure, for oil content no differences between sowing dates, nor interactions between sowing dates and selections were found. Therefore, prior to all other calculations, sowing dates were pooled.

Table 3.1. Number of evaluated plants ( $n$ ), population means ( $\bar{X}$ ), population variance ( $\sigma_p^2$ ), selection differential ( $S$ ) and response to selection ( $R$ ) in three cycles of mass selection for increased oil content in three populations (groups) of *Dimorphotheca pluvialis*.

Group	Cycle	n	Oil content				
			$\bar{X}$ <sup>1)</sup>		$\sigma_p^2$	S	R
A	C <sub>0</sub>	42	18.83	a	3.56		
	C <sub>1</sub>	70	19.16	a	4.57	4.62	0.33
	C <sub>2</sub>	67	19.68	ab	5.96	4.59	0.52
	C <sub>3</sub>	79	20.76	b	4.61	3.33	1.08
B	C <sub>0</sub>	44	18.63	a	5.90		
	C <sub>1</sub>	73	19.65	b	3.35	4.49	1.02
	C <sub>2</sub>	70	20.60	b	3.80	3.36	0.95
	C <sub>3</sub>	76	22.16	c	6.87	4.24	1.55
C	C <sub>0</sub>	34	21.09	a	9.02		
	C <sub>1</sub>	73	20.67	a	5.97	2.48	-0.42
	C <sub>2</sub>	70	21.39	ab	5.22	3.68	0.72
	C <sub>3</sub>	76	22.48	b	8.14	3.78	1.09

<sup>1)</sup> Population means (within groups) with same letters are not significantly different ( $P \leq 0.05$ ).

In other experiments with *D. pluvialis* in the Netherlands, Kreuzer *et al.* (1996) found that oil content was lower for an early sowing date. Van Dijk and Borm (1994) found the opposite, namely that early sowing had a positive effect on oil content. Both teams used *D. pluvialis* population A for their studies, but the experiments were carried out in different years. It seems that oil content in *D. pluvialis* is influenced by environmental factors. In the present trial however, the difference in growing conditions of the two sowing dates may have been too small to reveal such effects.

#### Selection response

The difference between the generation means was highly significant ( $P \leq 0.001$ ) in group B. In groups A and C the differences were less pronounced ( $P = 0.012$  and  $P = 0.052$ , respectively). In these groups, C<sub>0</sub>, C<sub>1</sub> and C<sub>2</sub> were not significantly different, but C<sub>3</sub> had a higher oil content than C<sub>0</sub> and C<sub>1</sub>. The results are listed in Table 3.1.

The response to selection, calculated from the difference between two successive generation means, varied between the cycles (Table 3.1). After the first cycle, only a significant response was found in group B. The negative response after the first cycle in group C should not be given much attention, since the C<sub>0</sub> and C<sub>1</sub> generations of this group were not significantly different, and, as a consequence, the response was not significant. In the second cycle in none of the groups the difference between the C<sub>1</sub> and C<sub>2</sub> generation was significant. In the third cycle some progress had been made, particularly in group B. The differences between any of the successive generations of groups A and C were not significant, but in both groups generation C<sub>3</sub> was significantly higher than C<sub>0</sub> (Table 3.1). Therefore, in all groups mass selection has resulted in an increase of oil content.

Table 3.2. Estimates of average response and realised heritability (and their standard errors), of three populations of *Dimorphotheca pluvialis*, after three cycles of mass selection, calculated by means of linear regression.

Group	Response		Realised heritability			
	regr. coeff. (b)	% v.a.f. <sup>1)</sup>	regr. coeff. (b)	% v.a.f. <sup>1)</sup>	t-test sign. <sup>2)</sup>	$h^2$
A	0.631 ± 0.121	89.7	0.134 ± 0.017	90.3	**	0.26
B	1.151 ± 0.096	97.9	0.290 ± 0.001	100.0	***	0.58
C	0.489 ± 0.245	49.9	0.077 ± 0.031	59.6	n.s.	0.15

<sup>1)</sup> % v.a.f. = % variance accounted for:  $100 \times (1 - (MS_{\text{res}})/(MS_{\text{tot}}))$

<sup>2)</sup> Level of significance of Student's *t*-test for *b* deviating from 0: \*\*\* =  $P \leq 0.001$ ; \*\* =  $P \leq 0.01$ ;

\* =  $P \leq 0.05$ ; n.s. = not significant.

Mass selection for increased oil content was particularly successful in group B. The average response per cycle was 1.15%, resulting in an increase of 3.5% after three selection cycles (Table 3.2). In group A and C the average increase in oil content per cycle was 0.63% and 0.49%, respectively. In the latter group, the regression line fitted the generation means rather poorly (49.9% variance accounted for). The larger response of group B could not be explained from the phenotypic variances of the base populations: in the year of selection the variances of populations A, B and C were 6.04, 5.61 and 3.26, respectively. Also, the range between highest and lowest value was largest in population A, 16.0%, with ranges of 13.5% and 10.8% for populations B and C, respectively. Since the selection procedures and conditions were equal for all three populations, the difference in response is most likely to be attributed to different genetic constitutions of the populations.

#### *Realised heritability*

From the slopes of the regression lines presented in Figure 3.1, it can clearly be seen that the realised heritability was largest for group B. In this population, the estimate of  $h^2$  was 0.58 (Table 3.2), while for group A and group C the realised heritabilities for oil content were 0.26 and 0.15, respectively. The regression coefficient (*b*) in group C did not deviate significantly from zero (Student's *t*-test,  $P=0.05$ ). As a consequence, the derived estimate for the realised heritability also was not significantly different from zero.

Heritability estimates are influenced by environmental circumstances (variation in culture or management) and the way phenotypes were measured (unit of measurement, number of measurements). Further, heritabilities are by definition population-specific: the additive genetic component of variance as well as the non-genetic component may vary among populations. Since the first two factors were equal for all populations in this experiment, it is likely that the observed wide range of heritability values for oil content in the three investigated populations can be predominantly attributed to differences in genetic constitution.

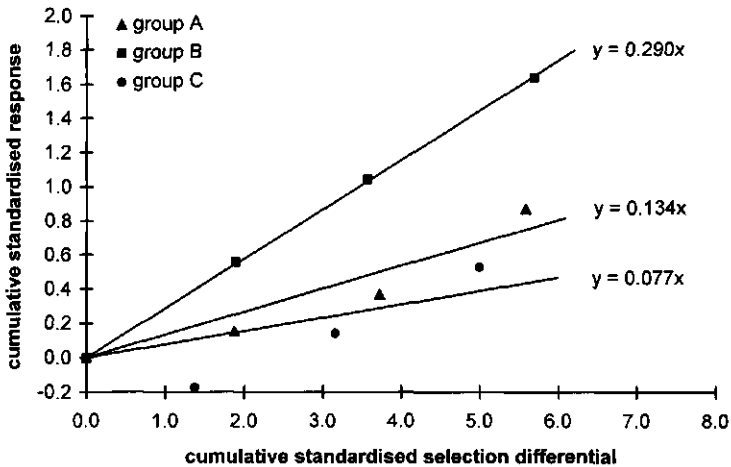


Figure 3.1. Regression of cumulative standardised response on cumulative standardised selection differential after three cycles of mass selection for oil content in three populations of *Dimorphotheca pluvialis*.

### Concluding remarks

The estimates for the realised heritabilities for oil content are specific for the populations used, selection procedures and environmental circumstances. The observed values for realised heritability in this experiment however, indicate that a substantial part of the observed phenotypic variance for oil content is additive genetic variance. Therefore, in general, (mass) selection for oil content in *D. pluvialis* will be effective. Considering the observed additive genetic variation in the base populations, an average oil content of at least 30% will be feasible.

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## Chapter 4

# Heritability of oil content and onset of flowering in a population of *Dimorphotheca pluvialis*

### Summary

To develop efficient breeding strategies for improvement of oil content and onset of flowering in *Dimorphotheca pluvialis* (L.) Moench, narrow-sense heritabilities of these characters were estimated. Forty plant progenies were tested in two years and heritabilities were estimated from parent-offspring regression as well as from half-sib family variance components analyses. For oil content, the heritabilities estimated by means of parent-offspring regression were 0.36 and 0.33 for 1993 and 1994, respectively. The heritabilities obtained from the variance components analyses were 0.41 and 0.23 for the two years. Heritability estimates for onset of flowering showed more variation. From parent-offspring regression the heritabilities were 0.72 and 0.37, and from variance component analysis the estimates were 0.53 and 0.19 for 1993 and 1994, respectively. The obtained heritability values and realised responses suggest that particularly in early selection generations even with mass selection considerable progress can be expected. Since correlation studies revealed neither phenotypic nor genotypic correlation between oil content and onset of flowering, selection for both characters can be performed independently.

### Introduction

World-wide, but particularly in Western Europe and the USA the interest in arable crops for non-food uses has substantially increased in the past few decades (Hatje 1989, Princen 1982 and 1983, Van Soest 1994a). Surpluses of the major food crops and the industrial interest for renewable resources have led to R&D programmes aiming at the introduction of crops with industrial applications (Meier zu Beerentrop and Röbbelen 1987, Princen and Rothfus 1984, Van Soest 1994b). In the USA, in the early 1960s, seed samples of hundreds of plant species were screened for chemical composition (Barclay and Earle 1974, Earle and Jones 1962, Jones and Earle 1966). In 1986, the Centre for Genetic Resources (CGN) in the Netherlands, now part of Plant Research International, started collecting and evaluating potential crops producing oils, fibres and carbohydrates. For both the agricultural potential and industrial interest, one of the most promising species was *Dimorphotheca pluvialis* (L.) Moench (Mulder *et al.* 1992, Van Soest 1994b). Its seeds contain about 21% oil with ca 60-65% dimorphecolic acid ( $\Delta^9$ -hydroxy, 10t, 12t-octadecadienoic acid), a unique fatty acid with a hydroxy group and two conjugated double bonds (Barclay and Earle 1965, Binder *et al.* 1964, Earle *et al.* 1964). This structure provides oil of *D. pluvialis* with unique properties, making it potentially suitable for application in pharmaceuticals, oleochemicals, coatings, fragrances and flavours (Derksen *et al.* 1993, Hayes *et al.* 1995, Muuse *et al.* 1992).

*D. pluvialis* is an annual, predominantly outcrossing species of the *Compositae* (*Asteraceae*) family, originating from southern Africa. The flower



heads are composed of female-fertile, male-sterile ray florets and hermaphrodite disk florets. Seeds (achenes) developing from disk florets are winged, those produced by the ray florets are slightly smaller and unwinged (Norlindh 1977). The percentage of oil, protein, pericarp, and the fatty acid composition is similar in both types of seed (Earle *et al.* 1964). Agronomic evaluation in the USA, Germany and the Netherlands revealed that seed yields are rather low and erratic, due to unsynchronised flowering and seed ripening, and extensive seed shattering (Hof and Nieboer 1994, Knowles *et al.* 1965, Meier zu Beerentrup and Röbbelen 1987, Willingham and White 1973). For an economically feasible production, oil content needs to be increased. In order to develop efficient breeding strategies for improvement of oil content and flowering synchronisation, studies on the heritability of these characteristics were undertaken. The results are described in this chapter.

## Materials and methods

### *Experimental*

The base material for this experiment was *D. pluvialis* population 883168, originating from a botanical garden in Dniepro Petrowsk (Ukraine), and chosen on account of its heterogeneous appearance in previous experiments. From an evaluation experiment with 220 widely spaced plants, 40 plants were chosen, representing a wide range in both oil content and onset of flowering. Progenies of the selected plants, being half-sib families, were sown in 1993 as well as 1994 to enable estimation of the heritability of the traits under study. The trials had a randomised block design with three replications. The experimental plots consisted of one row of eight plants. Plant spacing was 1 x 1 m, allowing harvesting of individual plants by hand. Seeds were collected at maturity, air dried, cleaned, separated into the two seed types and stored at room temperature. Since temperature is known to affect some stages of plant development (Kreuzer 1993, Kreuzer *et al.* 1996), onset of flowering was expressed as number of days (chronological time) as well as cumulative temperature (thermal time) between sowing and opening of the first flower. Opening of the first flower was recorded twice a week at regular intervals. Cumulative temperature was determined by summing the average daily temperatures (144 measurements/day) of the respective period, with a base temperature of 0 °C (Kreuzer 1993).

### *Determination of oil content*

Near Infrared Reflectance Spectrometry (NIRS) was used for rapid non-destructive determination of the oil content of the winged seeds. Oil content of unwinged seeds was not determined, since oil content of both types of seed is similar and winged seeds constitute the larger part of the yield (Earle *et al.* 1964, Meier zu Beerentrup and Röbbelen 1987). Seed samples were scanned and the reflectance (R) determined in a range of 100 - 2500 nm at 4 nm increments, using a Bran & Lübbe InfraAlyzer 500 spectrometer. Multiple linear regression was used to develop a calibration equation between

the near infrared reflectance data ( $\log(1/R)$ ) of 169 samples collected from three seasons, and the oil content determined by petroleum-ether extraction in Soxhlet equipment. With NIRS, the oil content of samples could be determined with a standard error of 1.3% (Groenendijk and Frankhuizen 1993).

#### Statistical procedures

Data of the experiments of 1993 and 1994 were subjected to ANOVA, using Genstat 5 procedures (Genstat 5 Committee 1989). Differences between families were tested using a nested analysis with three replications (blocks), families as plots and plants as subplots. Data were analysed on a yearly basis as well as combined. The combined data set was used to test the family by year interactions.

An estimate of narrow-sense heritability was made by means of linear regression of half-sib family means on performance of maternal parents:

$$h_{OP}^2 = \frac{2 \text{cov}(x,y)}{\text{var}(x)} = 2b_{OP} ,$$

where  $\text{cov}(x,y)$  is the covariance of the parental value ( $x$ ) and the phenotypic mean of the offspring ( $y$ ),  $\text{var}(x)$  is the phenotypic variance of the parents and  $b_{OP}$  is the regression coefficient of regression of offspring on parent (Falconer 1989). The standard error (s.e.) of the heritability estimate was calculated by doubling the standard error of the regression coefficient ( $b_{OP}$ ). To minimise scale effects between years, for the regression analyses all data were standardised by subtracting the mean and dividing the obtained value by the phenotypic standard deviation of the population in the respective years:

$$x' = \frac{x - \bar{x}_{pop}}{\sigma_{pop}} .$$

Estimates of narrow-sense heritabilities for oil content and onset of flowering were also calculated from the variance components, determined with residual maximum likelihood (REML) procedures of Genstat 5. The narrow-sense heritability is defined as the ratio of additive genetic variance ( $V_A$ ) to phenotypic variance ( $V_P$ ):

$$h^2 = \frac{V_A}{V_P} .$$

$V_A$  was estimated from the between-family variance:  $\sigma_f^2 = 1/4 V_A$ . The phenotypic variance,  $V_P$ , is the total of the between- and within-family variance (Falconer 1989). Therefore,

$$h_{VC}^2 = \frac{4\sigma_f^2}{\sigma_{TOT}^2} .$$

The ratio

$$\frac{\sigma_t^2}{\sigma_{TOT}^2}$$

is the intraclass correlation coefficient, symbolised by  $t$ . An approximate standard error of the heritability calculated from variance components, can be obtained by estimation of the variance of  $t$ . This variance is obtained by the so called 'delta technique', described by Bulmer (1985):

$$\text{var}(t) \cong \frac{2(1-t)^2 (t + (n-1)t)^2}{n(n-1)(s-1)},$$

where  $s$  and  $n$  are the number of families and the number of plants per family, respectively. Since it was assumed that  $h_{VC}^2 = 4t$ , the standard error of  $h_{VC}^2$  was approximated by

$$4\sqrt{\text{var}(t)}.$$

The realised response ( $R_r$ ) to selection for high and low oil content and early and late flowering was calculated by comparing the performance of the selected parental plants ( $\bar{x}_s$ ) in 1992, with the average performance of their corresponding offspring ( $\bar{y}_s$ ) in 1993 as well as 1994:

$$R_r = \bar{x}_s - \bar{y}_s.$$

Data were adjusted for year effects by subtracting the population mean of the respective year. Responses were calculated for 10%, 20% and 30% of parental plants selected.

A phenotypic correlation ( $r_p$ ) between oil content and onset of flowering was calculated for both years on the basis of individual plants. The observations were partially correlated since all plants belonged to specific families. Therefore, the respective family means were subtracted from the individual plant data to eliminate possible family effects. Subsequently, the phenotypic correlation was calculated per year as the ratio of the appropriate covariance to the product of the two standard deviations in the respective year:

$$r_p = \frac{\text{cov}(x, y)}{\sigma(x) \cdot \sigma(y)}$$

where  $x$  and  $y$  are the two characters under consideration.

The additive genetic correlation ( $r_A$ ) between the two characters was estimated from the parent-offspring relationship, as the additive genetic covariance of traits  $x$  and  $y$ , divided by the square root of the product of the

additive genetic variances of each character separately (Bos and Caligari 1995):

$$r_A = \frac{\text{COV}_A(x, y)}{\sqrt{\sigma_A^2(x) \cdot \sigma_A^2(y)}}$$

The additive genetic variance for trait  $x$  of a cross-fertilising species can be calculated from the parent-offspring covariance:

$$\sigma_A^2(x) = 2 \text{COV}(x_M, x_{HS})$$

The additive genetic variance of trait  $y$  is calculated in the same way. Analogously, the additive genetic covariance of cross-fertilising species is taken to be equal to twice the arithmetic mean of the covariance of  $x$  measured in maternal parents ( $M$ ) and  $y$  in offspring ( $HS$ ) and the covariance of  $y$  measured in maternal parents and  $x$  in offspring (Bos and Caligari 1995):

$$\text{COV}_A = \text{COV}(x_M, y_{HS}) + \text{COV}(y_M, x_{HS})$$

Thus, the additive genetic correlation can be expressed entirely in terms of phenotypic covariances:

$$r_A = \frac{\text{COV}(x_M, y_{HS}) + \text{COV}(y_M, x_{HS})}{\sqrt{2 \text{COV}(x_M, x_{HS}) \cdot 2 \text{COV}(y_M, y_{HS})}}$$

## Results

### Year effects

The average oil content in 1993 was slightly higher than in 1994, 20.5% and 19.8%, respectively (Table 4.1). Analysis of the combined years revealed significant differences between families ( $P \leq 0.001$ ) and no significant interaction between families and years. The plants grown in 1994 showed less variation in oil content than those grown in 1993. The range in oil content of the family means in both years was substantially smaller than the range observed in the parental population. The maximal difference in oil content between parental plants was 11.4%, while the family means showed a range of only 3.5% in 1993 and 2.8% in 1994.

Expressed as number of days, in 1993 plants started flowering a little later than in 1994, respectively 80.3 and 76.9 days after sowing (Table 4.1). Analyses of variance performed on the annual data sets revealed significant differences between families ( $P \leq 0.001$  in 1993, and  $P = 0.002$  in 1994). The variation measured in 1993 was considerably larger than in 1994, the range between family means was 12.0 days in 1993, and only 3.6 days in 1994 (Figure 4.1). Analysis of variance on the combined data set, showed a

Table 4.1. Population characteristics for oil content (% in winged seed) and onset of flowering (expressed as number of days (d) and cumulative temperature ( $^{\circ}\text{Cd}$ ) from sowing until first flower) of the parents in 1992, the offspring grown in 1993 and 1994, and the two year means. Presented data of the parents are based on individual plants, and those of the offspring on family means.

Characteristic	Parents	Offspring		
	1992	1993	1994	1993 + 1994
Oil content (%)				
Mean	21.0	20.5	19.8	20.1
Standard deviation	2.64	0.86	0.63	0.64
Range	14.9 - 26.3	18.8 - 22.3	18.3 - 21.1	18.6 - 21.3
Onset of flowering (d)				
Mean	77.1	80.3	76.9	78.6
Standard deviation	5.00	2.77	0.82	1.71
Range	68.0 - 89.0	75.0 - 87.0	75.1 - 78.7	75.1 - 82.6
Onset of flowering ( $^{\circ}\text{Cd}$ )				
Mean	1308.0	1229.3	1093.7	1161.5
Standard deviation	92.0	42.9	15.9	27.7
Range	1147-1528	1148-1335	1060-1129	1104-1228

significant interaction between families and years ( $P \leq 0.001$ ). This interaction was due to the difference in variation between both years, and disappeared after standardisation of the data.

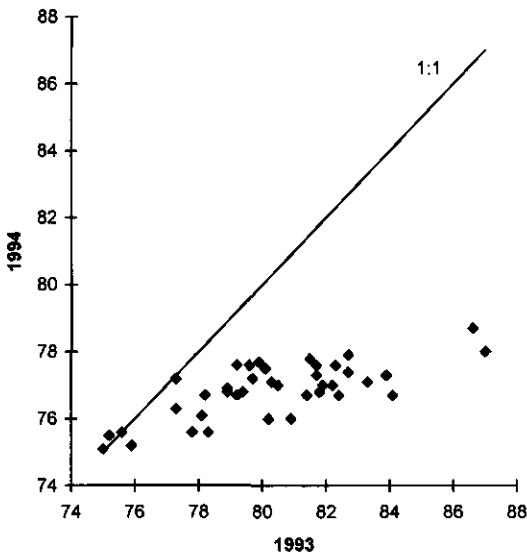


Figure. 4.1. Relationship between half-sib family means of 1993 and 1994 for onset of flowering (d).

When onset of flowering was expressed as cumulative temperature from sowing until opening of the first flower, a similar pattern was observed in the analyses of variance. Differences between families were significant in both years ( $P \leq 0.001$  in 1993, and  $P = 0.003$  in 1994), and the observed variation was still much larger in 1993 as compared to 1994 (Table 4.1). The average cumulative temperatures until flowering were 1229.3 °Cd and 1093.7 °Cd for 1993 and 1994, respectively. From the combined data set the interaction between families and years was significant ( $P = 0.004$ ). This was caused by a scale-effect, the interaction was not significant after standardisation of the data.

The observed variation for oil content and onset of flowering was remarkably different for 1993 and 1994, despite the fact that the same materials and procedures were used in both years, and experiments were carried out at the same location. However, the weather conditions were different in both years and this may have been an important factor. In the first ten weeks after sowing, in 1993 the daily temperatures were, on average, 1.6 °C higher than in 1994, resulting in a difference between years of 112 °Cd for this period. Then, in 1994 five weeks of continuing warm weather occurred, while temperatures remained moderate in 1993. In this period average daily temperatures were 15.9 °C and 21.1 °C for 1993 and 1994, respectively. However, the start of flowering was much later in 1993 as compared to 1994, both when expressed in chronological time and thermal time. Also the analyses of variance gave similar results for both units of measurement: more variation in 1993 as compared to 1994, and highly significant family by year interactions. Clearly, the differences in variation between years could not be explained solely by differences in temperature. Therefore, for comparison of families there was no apparent advantage of using cumulative temperatures over number of days and in all other calculations onset of flowering was expressed only as number of days from sowing until opening of the first flower.

#### *Heritability estimates by means of parent-offspring regression*

The heritability estimates for oil content by means of parent-offspring regression, were very similar; 0.36, 0.33 and 0.34 for 1993, 1994 and the combined data, respectively. For onset of flowering, parent-offspring regression using the annual data sets resulted in distinctly different estimates of heritability for both years, 0.72 and 0.37 for 1993 and 1994, respectively. Based on the combined data, the estimated heritability was 0.55 (Table 4.2).

Table 4.2. Heritability estimates ( $\pm$  s.e.) for oil content and onset of flowering from parent-offspring regression and variance components, based on offspring grown in 1993 and 1994.

Method	1993	1994	1993 + 1994
Oil content			
Parent-offspring regression	0.36 $\pm$ 0.11	0.33 $\pm$ 0.10	0.34 $\pm$ 0.09
Variance components analysis	0.41 $\pm$ 0.12	0.23 $\pm$ 0.08	0.27 $\pm$ 0.09
Onset of flowering			
Parent-offspring regression	0.72 $\pm$ 0.07	0.37 $\pm$ 0.08	0.55 $\pm$ 0.06
Variance components analysis	0.53 $\pm$ 0.14	0.19 $\pm$ 0.08	0.26 $\pm$ 0.09

Table 4.3. Mean squares and variance components for oil content in the analyses of half-sib families grown in 1993 and 1994.

Source of variation	1993	1994	1993 + 1994
Years	-	-	243.83
Blocks	33.53	111.77	72.65
Families	17.73***	9.38*	19.87***
Year by family	-	-	7.24 <sup>ns</sup>
Residual	5.56	5.21	5.39
Plants	4.41	2.57	3.49
VARIANCE COMPONENTS			
$\sigma_f^2$ <sup>1)</sup>	0.516	0.174	0.271
$\sigma_{yf}^2$	-	-	0.075
$\sigma_{ybf}^2$	-	-	0.191
$\sigma_{bf}^2$	0.083	0.302	-
$\sigma_{pl}^2$	4.408	2.566	3.489
$\sigma_{TOT}^2$	5.007	3.042	4.026

ns, \*, \*\*\* = Mean squares not significant, and significant at 5% and 0.1% probability levels, respectively.

<sup>1)</sup>  $\sigma_f^2$ ,  $\sigma_{yf}^2$ ,  $\sigma_{ybf}^2$ ,  $\sigma_{bf}^2$ ,  $\sigma_{pl}^2$ ,  $\sigma_{TOT}^2$  = Estimated variance components due to half-sib families, year by family, year by block by family, block by family interactions, plants and total, respectively.

Table 4.4. Mean squares and variance components for onset of flowering in the analyses of half-sib families grown in 1993 and 1994.

Source of variation	1993	1994	1993 + 1994
Years	-	-	5617.1
Blocks	155.5	15.0	85.3
Families	184.5***	16.3**	139.5***
Year by family	-	-	61.3***
Residual	48.4	7.7	28.0
Plants	34.9	7.2	20.8
VARIANCE COMPONENTS			
$\sigma_f^2$ <sup>1)</sup>	5.60	0.35	1.60
$\sigma_{yf}^2$	-	-	1.36
$\sigma_{ybf}^2$	-	-	0.83
$\sigma_{bf}^2$	1.61	0.05	-
$\sigma_{pl}^2$	34.89	7.21	20.79
$\sigma_{TOT}^2$	42.10	7.62	24.58

\*\* \*\*\* = Mean squares significant at 1% and 0.1% probability levels, respectively.

<sup>1)</sup>  $\sigma_f^2$ ,  $\sigma_{yf}^2$ ,  $\sigma_{ybf}^2$ ,  $\sigma_{bf}^2$ ,  $\sigma_{pl}^2$ ,  $\sigma_{TOT}^2$  = Estimated variance components due to half-sib families, year by family, year by block by family, block by family interactions, plants and total, respectively.

### Heritability estimates from components of variance

Variation for oil content was larger in 1993 as compared to 1994, as can be seen from the mean squares and variance components from half-sib family analysis (Table 4.3). Not only the between-family variance ( $\sigma_f^2$ ) and total variance ( $\sigma_{TOT}^2$ ) were larger for 1993, but also the ratio between both variance components was larger, resulting in a higher estimate of heritability. The heritabilities for oil content based on the data of 1993 and 1994 were 0.41 and 0.23, respectively. Based on the combined data, the estimated heritability was 0.27 (Table 4.2).

For onset of flowering the heritabilities for both years were rather dissimilar. As can be seen from Table 4.4, the total variance in 1994 was small, and the variance due to families very small, resulting in a low heritability value of 0.19 (Table 4.2). In 1993, the between family variance and the total variance were both much larger, as well as the ratio between both variance components, resulting in a higher heritability value of 0.53. From the combined dataset, the estimated heritability was 0.26.

### Realised response to selection

The realised responses to selection for high and low oil content and early and late flowering, calculated for 10%, 20% and 30% of the parental plants selected, are presented in Table 4.5. As expected, the response to selection, in general, decreased with an increasing fraction of plants selected. For oil content, selection towards a high oil content showed a better response than selection towards a low one. Particularly with regard to increased oil content, the response to selection was substantially larger in 1993 than in 1994. Based on the combined results of 1993 and 1994, in this population, with 10% of the parental plants selected, an increase of oil content with 0.8% was achieved in one selection cycle.

For onset of flowering a similar pattern was observed; a reduced response with an increased number of selected plants, and a much larger response in 1993 than 1994. In 1993, the response to selection for early and late flowering was similar, but in 1994 the response to selection for early flowering was better than for late flowering. In this population, based on the combined results of 1993 and 1994 and with 10% of the parental plants selected, the response to selection in either direction was ca 2.5 days.

Table 4.5. Realised response ( $R_s$ ) for high and low oil content and early and late flowering at different selection intensities, based on performance of selected parents in 1992 and corresponding offspring in 1993 and 1994.

Selection	Fraction selected (%)	$R_s$	
		1993	1994
Oil content (%)			
High	10%	0.99	0.51
	20%	0.80	0.50
	30%	0.52	0.44
Low	10%	0.22	0.33
	20%	0.25	0.24
	30%	0.38	0.25
Onset of flowering (d)			
Early	10%	3.64	1.16
	20%	3.61	0.97
	30%	2.98	0.64
Late	10%	4.50	0.63
	20%	3.32	0.39
	30%	2.88	0.46



### *Phenotypic and genotypic correlation*

The phenotypic correlation coefficients ( $r_p$ ) between oil content and onset of flowering based on individual plants were 0.182 and 0.124 for 1993 and 1994, respectively. Although these values are statistically significant ( $df=960$ ), they have no practical relevance.

The additive genetic correlations ( $r_A$ ), based on the relationship between parents and offspring ( $df=40$ ), were very low and not significant: -0.156 and 0.044 for 1993 and 1994, respectively. Oil content and onset of flowering seem to be uncorrelated characters in *D. pluvialis*.

## **Discussion**

### *Year effects*

The results showed that, in 1993, flowering started later, and the observed variation was much larger in 1993 than in 1994. Comparison of chronological time with thermal time of the period from sowing to flowering, indicated that differences in temperature could not entirely explain this discrepancy between years. This is in accordance with earlier publications on the influence of temperature on development of *D. pluvialis* (Kreuzer 1993, Kreuzer *et al.* 1996). Kreuzer and co-workers found that the germination phase was highly affected by temperature, and a cumulative temperature of ca 100 °Cd was required from sowing until plant establishment. The period from establishment until flower initiation was, however, less sensitive to temperature, and appeared to take an average time of ca 32 days. Only at very high temperatures (> 20 °C) this phase was prolonged by several days, indicating some sort of vernalisation requirement. Unfortunately too few data on the last phase from flower initiation until opening of the first flower were available to draw reliable conclusions. However, preliminary results indicated that duration of this phase was again temperature dependent: higher temperatures seemed to accelerate this process. Although in the experiment described in this paper no observations on the separate stages of development were available, several remarks can be made. The 100 °Cd required for germination was reached after five days in 1993 and eight days in 1994. During the vegetative phase average daily temperatures stayed far below 20 °C, so bearing in mind the findings of Kreuzer, it is likely that this phase lasted the same time in both years, despite differences in temperature. On average, plants started flowering 3.5 days earlier in 1994, despite the presumed three days delay during germination. Since temperatures from ten weeks after sowing in 1994 were substantially higher than in 1993, and the earliest plants started to flower after ca 9.5 weeks, the hypothesis that the phase from flower initiation until opening of the first flower is enhanced by higher temperatures can be supported. However, this relationship probably is not linear, since the cumulative temperature from presumed flower initiation until average opening of the first flower was ca 665 °Cd in 1993, and only 560 °Cd in 1994. In conclusion, although temperature may play a role in some stages of development of *D. pluvialis*, the entire period from sowing until opening of the

first flower can most likely not simply be expressed in terms of a cumulative temperature.

The summer of 1993 was not only characterised as rather cool, but also as cloudy with abundant rainfall. In 1994, during the first five weeks of flowering and seed set, the weather was sunny, very warm with practically no rain. It is likely that these exceptionally warm and dry weather conditions in 1994 led to early maturation of the plants. As can be seen from Figure 4.1, the reduced variation for onset of flowering in 1994 is due to the relatively early flowering of late families. In fact, there was a positive relationship between onset of flowering in 1993 and the deviation from the expected flowering date in 1994 (1:1 line). This was true when onset of flowering was expressed in chronological time as well as thermal time, indicating that early maturation of plants in 1994 may have been due to environmental stress other than temperature, such as drought. Early maturation could also explain the somewhat lower, and less variable oil content observed in 1994.

Differences in population variance of parents (based on individual plants) and progenies (based on family means) are to be expected. Still, the limited variation between individual plants as observed in 1994, compared to the relatively large variation in the parental population, underlines the influence of environmental conditions on the observed characters, and stresses the importance of standardisation of data when comparing parents and progenies.

#### *Heritability estimates and response to selection*

The heritability values for oil content estimated from parent-offspring regression are more or less in accordance with the estimates based on variance components: for 1993 the estimation from parent-offspring regression was slightly lower, for 1994 and the combined data somewhat higher. Though the estimated heritabilities for oil content from both methods are moderately low, a substantial part of the variance is additive, as can be seen from the variance components. Therefore, considerable progress may be expected from selection. Various authors have reported on moderate to high heritabilities for oil content of industrial crops: 0.70 for *Crambe abyssinica* Hochst. ex R.E. Fries (Lessman 1975), 0.65 for *Euphorbia lathyris* L. (Hondelmann 1987), 0.69 for cotton (Akdemir *et al.* 1986), 0.52 for sunflower (Fick 1975) and 0.67 - 0.92 for safflower (Vijayakumar and Giriraj 1985). The moderate heritabilities for oil content in *D. pluvialis* from the present experiment are in accordance with results from other experiments in which realised heritabilities of 0.15 - 0.58 were found in three populations of *D. pluvialis* after three cycles of mass selection (Chapter 3).

Taking both years and estimation methods into account, the results of the present experiment show a large discrepancy in the heritability estimates for onset of flowering, ranging from 0.19 to 0.72. It is possible that the heritabilities based on the performance of the families tested in 1994 are estimated relatively low because of the limited variation for onset of flowering in that year. The time span between individual plants for onset of flowering was only 21 days, and the range between the family means was only 3.6

days. Therefore, the scoring interval of  $\approx 3.5$  days (twice per week), was relatively large compared to the observed variation. This most likely has affected the within-family variance negatively, and hence the estimation of the additive genetic variance, as well as the estimation of the heritability from variance components. It also may have slightly influenced the value of the family means in either direction, which may have had a relatively large effect on the relationship between parents and family means of 1994 and, as a result, the heritability estimated by means of parent-offspring regression. Therefore, the estimates of heritability based on the families of 1993 seem more realistic. These moderate to high values are in accordance with heritability estimates for onset of flowering in other industrial crops: 0.78 in *Crambe abyssinica* (Lessman 1975), 0.98 in *Euphorbia lathyris* (Hondelmann 1987), 0.36 - 0.73 in *Limnanthes alba* Hartw. (Jain 1979) and 0.61 - 0.75 in pearl millet (Sandhu and Phul 1984).

The two methods of estimation of heritability are based on different principles. The parent-offspring regression method is based on the resemblance between maternal parents and corresponding offspring, while the estimate from variance components is based on the variation between half-sib families relative to the total variance. The parent-offspring method directly compares the performance of maternal parents and their offspring and therefore gives an indication of how well a character is passed on to the next generation. However, parents and offspring are generally assessed in different years, and genotype by year interaction can be a disturbing factor. If this interaction does not influence ranking but is merely a scale effect, it can be minimised by standardisation of the data, as was done in the described experiment. Another weakness of the regression of half-sib offspring on one parent is that relatively much weight is given to one single phenotypic value of the individual parents. The impact of errors in determination of these phenotypic values can be large when the number of parents is small, and the variation in the parental population is limited. The estimation of heritability by means of variance components is based on one generation, the offspring, and therefore not subject to genotype by year interactions.

In the conducted experiment the variation in the parental population was relatively large for both traits, and the size of the parental population ( $n=40$ ) as well the number of offspring per family ( $n=24$ ) were adequate for a reliable estimation of heritability by means of both methods. This was reflected in the rather small, and moreover similar standard errors of the heritability estimates from both methods (Table 4.2).

The response to selection for low oil content was smaller than for high oil content. Asymmetrical responses are often found, and possible causes are comprehensively discussed by Falconer (1989). The lower responses in 1994 as compared to 1993, both for oil content and onset of flowering, are most likely due to the reduced variation in that year. For reasons explained before, responses based on the data of 1993 are probably more realistic. Provided environmental conditions allow for expression of variation, oil content and onset of flowering will respond readily to mass selection. For onset of flowering, the accuracy of the realised response can be improved by increasing the frequency of observations to 3-4 times per week. The observed

response for increased oil content of 0.8% at a selection intensity of 10%, is in accordance with results of other experiments with *D. pluvialis*, where an average increase of 0.5 - 1.2% in oil content was obtained in three populations of *D. pluvialis* subjected to mass selection at the same selection intensity (Chapter 3).

## Conclusions

In the currently available populations, which are rather heterogeneous, mass selection for onset of flowering and oil content in *D. pluvialis* will be effective, particularly when selection pressure is high and environmental conditions allow for expression of variation. A substantial part of the observed variation for both characters was additive, indicating that progress may still be expected in later selection generations, provided that ample effective population size and selection intensities allow for genetic variation. Since heritabilities tend to decrease in the course of selection, particularly for oil content other methods than mass selection, for example family selection, should be considered. The absence of a phenotypic and genotypic correlation between both characters suggests that when selection for either oil content or onset of flowering is performed, no undesired correlated response for the other character is expected.

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## Chapter 5

# A model describing the flowering of single plants, and the heritability of flowering traits of *Dimorphotheca pluvialis*

### Summary

In the development of new crops such as *Dimorphotheca pluvialis* (L.) Moench, improvement of flowering synchronisation is an important breeding objective. The flowering of single plants of *D. pluvialis* could be described by a logistic curve obtained by the regression of cumulative number of open flowers on time. The curve is characterised by three parameters, corresponding with the total number of flowers produced by the plant, the rate of flowering development and the day at which peak bloom is reached. From these parameters two other characteristics were derived, i.e., onset of flowering and duration of flowering. The use of the flowering model for selection for improved flowering synchronisation is discussed. Heritabilities of flowering traits were estimated using parent-offspring regression and variance components analyses. Onset of flowering and date of peak bloom showed high ( $>0.69$ ), and total number of flowers moderate to high (0.30 - 0.90) heritability values, indicating that for these traits considerable progress may be expected from mass selection, particularly in the early selection generations. Duration of flowering showed low to moderate values (0.25 - 0.45), and methods other than mass selection (e.g. family selection) should be considered. Determination of phenotypic and genetic correlations revealed only an additive genetic correlation between date of peak bloom and duration of flowering ( $r_A = 0.80$  and  $0.69$  for 1993 and 1994, respectively), suggesting the possibility of indirect selection for curtailed duration of flowering by means of selection against late date of peak bloom. Duration of flowering, total number of flowers and onset of flowering were not correlated.

### Introduction

In the past few decades, structural surplus production of arable food crops in Europe has led to the search for alternative land uses (Rexen 1992). One of the options under consideration is growing crops for the production of renewable resources for industrial use. In the Netherlands, since 1986 several R&D programmes have been initiated, partly funded by the Dutch government and the European Community. In the framework of these multidisciplinary programmes, the former Centre for Plant Breeding and Reproduction Research (CPRO-DLO, now part of Plant Research International), was responsible for the introduction, evaluation and breeding research of new crops with potential industrial applications. Considering both the industrial interest and agricultural potential, *Dimorphotheca pluvialis* (L.) Moench was regarded as one of the most promising species (Mulder *et al.* 1992, Van Soest 1994). The principal constituent of its seed oil is dimorphecolic acid:  $\Delta^9$ -hydroxy,10t,12t-octadecadienoic acid (Binder *et al.* 1964, Earle *et al.* 1964). The highly reactive conjugated hydroxydiene structure makes it a versatile raw material for specialty applications in e.g. the oleochemical, pharmaceutical and flavour and fragrances industry. After chemical modification, a wide variety of new chemicals can be obtained which can be

used as compounds in lubricants, surface coatings, plastic foams and nylons (Derksen *et al.* 1993, Knowles *et al.* 1965, Muuse *et al.* 1992). The use of dimorphecolic acid in the production of pheromones is now ready for commercialisation (J.T.P. Derksen, pers. comm.).

*D. pluvialis* is a herbaceous annual, native to south-west Africa. (Norlindh 1946 and 1977). Plants differ considerably in growth habit, ranging from erect to procumbent. Depending on growth habit, plants are 10-50 cm in height. In general, one main stem is formed with several branches. Particularly the basal branches are strongly ramified. All end meristems sooner or later turn generative and form flower heads. As is common in *Asteraceae*, the flowers consist of a capitulum bearing two types of florets. In the species *D. pluvialis*, the ray florets have one large white petal, which is often coloured purple at the base, giving the appearance of a 'ring' in the flower. Disk florets are yellow, purple or have an intermediate colour. In the genus *Dimorphotheca*, the disk florets are hermaphrodite, and the ray florets are female fertile but male sterile. Both types of florets produce distinctly different types of seeds (achenes). Seeds produced by the ray florets are rod-shaped, while those produced by the disk florets are flattened with winged margins (Norlindh 1943 and 1977, Barclay and Earle 1965). Both types of seed contain oil in similar quantities and of equivalent fatty acid composition (Earle *et al.* 1964).

The species seems well adapted to the maritime climate of north-west Europe, and fits in a rotation system with annual crops. In the Netherlands it is sown in April, and can be harvested in August (Van Dijk and Borm 1993). The potential seed yield amounts up to 2500 kg/ha, but in practice yields are erratic because of a long, unsynchronised flowering period combined with a poor seed retention. Previous experiments showed little variation for seed retention (Chapter 1). Moreover, the observed variation seemed to be primarily caused by differences in environmental conditions, overshadowing possible genetic differences. Therefore, attention was focused on improvement of flowering synchronisation. For this, two main components were distinguished: the synchronisation between, and the synchronisation within plants. Synchronisation between plants is attained when plants of a population flower at the same time. Synchronisation within a plant is achieved when its flowers are produced in a short period of time. Both components are considered important for improvement of flowering synchronisation of a population (Hof 1996). Therefore, the flowering process of *D. pluvialis* plants in time was studied, and the variation for flowering characteristics determined. Furthermore, to facilitate optimisation of breeding strategies for improved flowering synchronisation, heritabilities of flowering characteristics were estimated.

## Materials and methods

### *Plant material and experimental layout*

For the experiments, seed of *D. pluvialis* population 879585 was used as base material. This population originated from a botanical garden in Dniepro

Petrowsk (Ukraine), and was chosen on account of its heterogeneous appearance in previous experiments. In 1992, a trial was sown with 200 plants of this population. Plant spacing was 1 x 1 m, allowing assessment of individual plants. When plants started flowering, the open flowers were counted twice a week at regular intervals and counting continued until plants stopped flowering. As soon as flowering had ended, plants were gently lifted and the total number of heads per plant was determined. Flowering traits were determined using a non-linear regression model describing the flowering of individual plants, which will be explained in the next section.

From this population, 20 plants were selected representing a wide range in variation for flowering characteristics. Progenies of these plants, being half-sib families, were tested in 1993 as well as 1994. Each year, ten plants of each of the 20 families were sown using a completely randomised block structure with ten replications. Within replications (blocks) each family was represented by one plant. Plant spacing was 1 x 1 m. Data were collected, and flowering traits were determined following the same procedures as in 1992. All trials were located at the CPRO-DLO experimental farm in Lelystad, the Netherlands.

#### *Flowering model*

Flowering of individual plants was studied by counting the open flowers at regular intervals. It was found that the cumulative number of open flowers per plant per counting date ( $y$ ) plotted against time ( $x$ ), fitted a logistic curve:

$$y = \frac{C}{1 + e^{-B(x-M)}} \quad (1)$$

In this curve,  $x$  is the time span from sowing until the respective counting date.  $B$  is the so-called 'slope parameter'.  $M$  is the date corresponding with the inflexion point of the curve, it is the date at which the number of open flowers is at its maximum (peak bloom).  $C$  represents the upper asymptote (total number of flowers). The total number of flower heads determined after flowering had ended, was for most plants substantially smaller than the cumulative number of open flowers at the last counting date. This discrepancy most likely was caused by the fact that open flowers were counted repeatedly, at successive counting dates. Observations from other experiments (data not shown), where individual flowers were open for 4-8 days, support this hypothesis. Since  $C$ , the upper asymptote, should represent the actual total number of flowers produced by the plant, it was decided to introduce an 'adjustment factor' ( $AF$ ), being the ratio of the number of flower heads determined after flowering ( $NHAF$ ) and the cumulative number of flowers at the last counting date ( $CNF$ ):

$$AF = \frac{NHAF}{CNF}$$



The adjustment factor was determined for each individual plant, and ranged between 0.5 and 1.0, depending on the duration of individual flowers being open. The cumulative numbers of open flowers per counting date were all multiplied by the adjustment factor prior to curve fitting. By doing so, the actual increase in number of flowers between two consecutive counting dates could be determined, and the cumulative number of open flowers at the last counting date was equal to the actual total number of flowers produced by the plant. Parameter  $C$  of equation 1 was therefore substituted by  $C'$ :

$$C' = C \times AF.$$

It appeared that the duration of individual flowers being open was largely determined by genotype and showed no systematic deviations within the total flowering period (data not shown).

From the curves, two other flowering characteristics of individual plants were derived: onset of flowering and duration of flowering. Duration of flowering was defined as the time interval in which the plant showed open flowers. However, since a logistic curve is bordered by asymptotes, it is not possible to calculate the time required by a plant to open all of its flowers (the 100% -interval) directly from the curve. From earlier observations it was found that the time lapse between the opening of the first and second flower was in many plants relatively long compared to the remainder of the flowering process, and it would be more appropriate to consider flowering had started when at least two flowers were open. To enable a fair comparison between plants differing in total number of flowers, it was decided to express onset of flowering relative to the total number of flowers produced by the plant. Since a vast majority of the plants (98%) produced more than 80 flowers, onset of flowering of a plant was defined as the date at which 2.5% of its total number of flowers was open. For calculation of onset and duration of flowering, equation 1 was rewritten as a function of  $y$ :

$$x = M + \frac{\ln\left(\frac{C'}{y} - 1\right)}{-B} \quad (2)$$

After curve-fitting, the onset of flowering was subsequently calculated by substitution of  $y = 0.025C'$  (2.5% of the total number of flowers) in equation 2:

$$x_{2.5\%} = M + \frac{\ln\left(\frac{C'}{0.025C'} - 1\right)}{-B} = M - \frac{\ln(39)}{B} \quad (3)$$

Duration of flowering was analogously defined as the time interval between 2.5% and 97.5% of the total number of open flowers:

$$x_{97.5\%} - x_{2.5\%} = \left[ M + \frac{\ln(39)}{B} \right] - \left[ M - \frac{\ln(39)}{B} \right] = 2 \left[ \frac{\ln(39)}{B} \right] = \frac{2}{B} \ln(39) \quad (4)$$

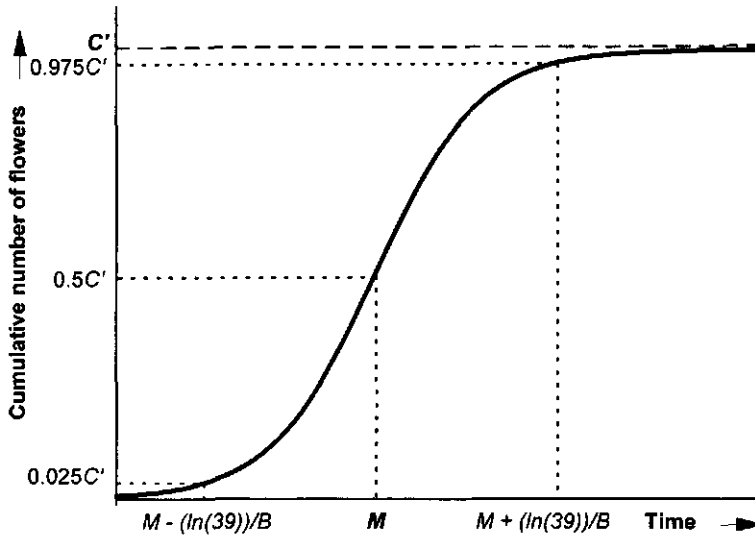


Figure 5.1. Logistic curve describing course of flowering of individual plants of *D. pluvialis*, with representation of parameters  $C'$  (total number of flowers) and  $M$  (date of peak bloom), onset of flowering at 2.5% ( $y = 0.025C'$ ) and duration of flowering (95% interval between  $y = 0.025C'$  and  $y = 0.975C'$ ).

In Figure 5.1, an example of a logistic curve is presented, with representation of the parameters  $C'$  and  $M$ , onset of flowering and duration of flowering.

Since temperature is known to affect some stages of plant development in *D. pluvialis* (Kreuzer 1993, Kreuzer *et al.* 1996), time was not only expressed as number of days (chronological time), but also in terms of cumulative temperature (thermal time). Cumulative temperature was determined by summing the average daily temperatures (144 measurements/day) of the respective period, with a base temperature of 0 °C (Kreuzer 1993). All parameters were estimated a second time by plotting number of flowers against cumulative temperatures, and fitting the same model as described above. For curve-fitting, Genstat non-linear regression procedures were used (Genstat 5 Committee 1989).

#### Data analyses and heritability estimates

Data of the experiments conducted in 1993 and 1994 were subjected to ANOVA using Genstat 5 (Genstat 5 Committee 1989). The model used for the ANOVA was:

$$Y = \mu + \text{Year} + \text{Year} \cdot \text{Block} + \text{Family} + \text{Year} \cdot \text{Family} + \text{Residual}$$

Year mean squares were tested against Year.Block mean squares for a crude estimation of year effects. Year.Family interaction was tested against the Residual.

Narrow-sense heritabilities for flowering characteristics were estimated by means of linear regression of half-sib family means on maternal parent (Falconer 1989). The standard error (s.e.) of the heritability estimate was calculated by doubling the standard error of the regression coefficient. To minimise scale effects between years, prior to the regression analyses all data were standardised by subtracting the mean and dividing the obtained value by the phenotypic standard deviation of the population in the respective years.

Heritabilities were also estimated from variance components, determined with residual maximum likelihood (REML) procedures of Genstat 5. Variance components were calculated per year as well as from the combined years. The model used for the combined analyses was:

$$Y = \mu + \text{Year} + \text{Year.Block} + \text{Family} + \text{Year.Family} + (\text{Year.Block.Family} + \text{error})$$

In these analyses, Year and Year.Block components were considered fixed, and the other components were considered random. In the analyses per year, the same model was used, omitting the Year term. The narrow-sense heritability on individual plants basis is defined as the ratio of additive genetic variance ( $V_A$ ) to phenotypic variance ( $V_P$ ).  $V_A$  was estimated from the between family variance:  $\sigma_f^2 = 1/4 V_A$ . The total phenotypic variance,  $V_P$ , is the sum of the variance due to between and within-family components (Falconer 1989). An estimate of the standard error was obtained by estimation of the variance of the intraclass correlation coefficient (Bulmer 1985). The procedures on the calculations of heritabilities and their standard errors have been described in detail in Chapter 4.

Phenotypic correlations ( $r_P$ ) between flowering characteristics were calculated on the basis of the family means of 1993 and 1994, from the appropriate covariances and standard deviations of the traits under consideration. Additive genetic correlations ( $r_A$ ) between two characters were estimated from phenotypic covariances:

$$r_A = \frac{\text{COV}(x_M, y_{HS}) + \text{COV}(y_M, x_{HS})}{\sqrt{2 \text{COV}(x_M, x_{HS}) \cdot 2 \text{COV}(y_M, y_{HS})}}$$

where  $x$  and  $y$  are the traits under consideration and the suffixes  $M$  and  $HS$  refer to maternal parent and half-sib offspring, respectively (Chapter 4, Bos and Caligari 1995).

## Results and discussion

### Experiments

In the experiment of 1993, germination conditions were not optimal and at eleven plant positions no plant established. Three weeks after sowing it was decided to resow these positions, in order to minimise the number of missing plants. These plants were discarded from calculations on date of onset of flowering and date of peak bloom.

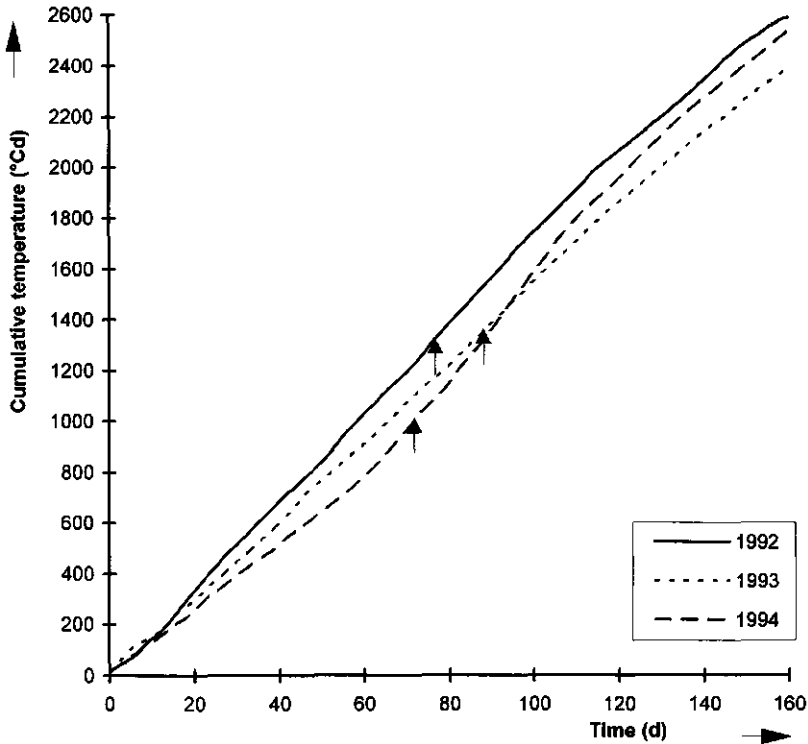


Figure 5.2. Cumulative temperature in the growing seasons of 1992, 1993 and 1994. The arrows mark the average onset of flowering of the plants in these years.

In 1993 as well as 1994 some plants wilted and perished during the growing season. Closer inspection revealed that they suffered from *Sclerotinia sclerotiorum* (Lib.) de Bary. Incidentally, rust (*Puccinia* spp.) and downy mildew (*Bremia* spp.) were observed on leaves and grey mould (*Botrytis cinerea* Pers. ex Fr.) on flower heads, but no severe attacks of pests or diseases occurred.

#### The flowering model

The used logistic model, based on the cumulative numbers of open flowers, described the flowering of individual plants very well. For the data of 1992, 1993, and 1994, the model on average explained 99.7%, 99.9%, and 99.7%, respectively, of the observed variation. In terms of explained variation, it made no difference whether time was expressed as number of days or as cumulative temperature. Most likely, this was caused by the fact that the average daily temperatures during the flowering period were relatively constant (Figure 5.2).

Table 5.1. Population characteristics of the parents grown in 1992 (n=20) and their offspring grown in 1993 and 1994 (n≈200). Parameters based on regression with time expressed as number of days (d).

Parameter	Year	Average	Standard deviation	Minimum	Maximum	CV% <sup>1)</sup>
Onset of flowering	'92	76.6	5.7	67.4	84.9	-
	'93	86.1	6.6	68.0	108.1	6.2
	'94	73.6	4.3	63.0	84.4	5.4
Slope, <i>B</i>	'92	0.273	0.100	0.144	0.544	-
	'93	0.198	0.055	0.103	0.404	25.9
	'94	0.247	0.045	0.131	0.470	18.0
Date of peak bloom, <i>M</i>	'92	91.8	8.4	77.9	103.2	-
	'93	105.6	8.6	89.2	135.6	7.0
	'94	89.0	4.4	79.9	100.8	4.5
Number of flowers, <i>C'</i>	'92	342.5	196.2	104.8	790.4	-
	'93	437.1	179.6	58.1	1139.0	39.8
	'94	215.5	90.8	29.4	549.7	36.7
Duration of flowering	'92	30.3	10.8	13.5	50.7	-
	'93	39.7	10.2	18.2	71.1	24.7
	'94	30.8	6.0	15.6	55.8	18.3

<sup>1)</sup> Coefficient of variation:  $\frac{\sqrt{MS_{res}}}{\bar{x}} * 100\%$

Table 5.2. Population characteristics of the parents grown in 1992 (n=20) and their offspring grown in 1993 and 1994 (n≈200). Parameters based on regression with time expressed as cumulative temperature (°Cd).

Parameter	Year	Average	Standard deviation	Minimum	Maximum	CV% <sup>1)</sup>
Onset of flowering	'92	1308.0	110.2	1125.9	1470.2	-
	'93	1334.6	119.7	1027.5	1838.6	6.9
	'94	1000.3	80.6	808.9	1213.7	7.3
Slope, <i>B</i>	'92	0.015	0.001	0.008	0.029	-
	'93	0.013	0.003	0.007	0.024	23.5
	'94	0.012	0.002	0.007	0.024	18.9
Date of peak bloom, <i>M</i>	'92	1577.7	152.0	1324.0	1782.1	-
	'93	1642.1	138.8	1370.0	2199.1	6.9
	'94	1314.5	92.9	1133.4	1584.4	6.4
Duration of flowering	'92	539.4	185.7	248.8	927.1	-
	'93	617.4	140.3	303.4	1018.4	21.4
	'94	628.4	124.9	303.8	1124.2	18.7

<sup>1)</sup> Coefficient of variation:  $\frac{\sqrt{MS_{res}}}{\bar{x}} * 100\%$

### Year effects

Differences between years were highly significant ( $P \leq 0.001$ ) for all parameters. In 1993, plants started flowering almost two weeks later than in 1994, and ten days later than in 1992 (Table 5.1). The minimum values show that there was not much difference between the onset of flowering of the earliest plants of all years, but particularly in 1993 several plants started flowering much later as compared to the other years. Not surprisingly, date of peak bloom was also later in 1993 as compared to 1994, namely 16 days. Again, the difference between years in date of peak bloom of the earliest plants was relatively small (only ten days), compared to the differences between years in date of peak bloom of the latest plants (more than 30 days). Clearly, variation for onset of flowering and date of peak bloom was larger in 1993 than in 1992 and 1994. The total number of flowers also differed substantially between years. In 1993, plants produced on average 437 flowers, against 216 in 1994. This large contrast between years suggests that flower production may be greatly influenced by environmental conditions, and it is possible that flower production in 1994 was limited because of early maturation of plants. Conversely, in 1993 the cool and wet weather conditions may have hampered insect pollination, which was earlier established as an important factor determining seed set (Chapter 2). Although no data on seed set are available from this experiment, it is possible that reduced seed set prolonged the flower production. The standard deviations show that in 1994 the variation for number of flowers was much less than in 1992 and 1993. The average duration of flowering in 1993 was 40 days, almost ten days longer than in the other years.

In terms of thermal time, onset of flowering and date of peak bloom showed similar results to the observations found for chronological time (Table 5.2). The difference in average onset of flowering between 1993 and 1994 was  $334^\circ\text{Cd}$ , and for date of peak bloom  $328^\circ\text{Cd}$ . It seems that temperature is not the most important factor determining the period from sowing until onset of flowering and date of peak bloom. Duration of flowering however, was equivalent in 1993 and 1994 when expressed in thermal time:  $617$  and  $628^\circ\text{Cd}$ , respectively. From Figure 5.2 it can be seen that in this phase of plant development temperatures were much higher in 1994 as compared to 1993. Clearly, these higher temperatures shortened the flowering period of individual *D. pluvialis* plants. The large variation between plants however, suggests that duration of flowering is also influenced by other, yet undetermined, factors than temperature.

In general, the variation of the observed parameters was much larger in 1993 as compared to 1994. Since the same seed material was used in both years, it is likely that the difference in variation was due to environmental conditions. Comparison of the parameters based on chronological time to the parameters based on thermal time, showed that only for duration of flowering the discrepancy between years could most likely be attributed to differences in temperature. For all other parameters, temperature was not the most important factor. This confirms the findings of Kreuzer and co-workers (Kreuzer 1993, Kreuzer *et al.* 1996), who found that although the germination

phase was determined by cumulative temperature, the period from seedling to first flower was most likely only partially controlled by temperature.

Analyses of variance showed that for all parameters the differences between families were highly significant ( $P \leq 0.001$ ). For onset of flowering and date of peak bloom a significant year by family interaction was observed, both when expressed in chronological time or thermal time. This interaction apparently was due to a scale effect, caused by the observed difference in variation of the parameters between years. For total number of flowers, slope and duration of flowering such interactions were not found.

With the exception of duration of flowering, the obtained results indicate that there was no apparent advantage of using thermal time. Therefore, for all other calculations the parameters based on chronological time were used. Duration of flowering was expressed both in terms of days and cumulative temperature.

#### *Heritability estimates*

For all examined parameters it was found that in the variance components analyses of the combined data, the Year.Family component was very small, indicating that there was hardly any added variation due to years on top of the within family variation. The heritability values for onset of flowering were rather high (0.69 - 1.50), both from estimation by means of parent-offspring regression and from variance components (Table 5.3). For both methods, heritability estimates based on the data of 1993 were higher than those based on the data of 1994. The very high estimate based on variance components of the data of 1993 was due to the high value of the between-family component. It is possible that non-genetic components were included in the between-family variance, causing overestimation of the additive genetic variance. Also, since early flowering families may have experienced environmental conditions different from those experienced by late flowering families, genotype by environment interactions cannot be ruled out. A similar pattern in heritability estimates was observed for peak bloom, which was expected since onset of flowering and date of peak bloom are correlated parameters (see equation 3). In previous experiments using other plant material of *D. pluvialis*, heritability estimates of 0.53 (from variance components) and 0.72 (from parent-offspring regression) were reported for onset of flowering (Chapter 4). The obtained values suggest that in *D. pluvialis* quick progress may be expected from mass selection for onset of flowering or date of peak bloom.

Moderate heritabilities were found for total number of flowers. When estimated by means of parent-offspring regression, heritability values were similar for both years (0.51 - 0.52). However, from the variance components, the heritability estimate for 1994 was higher (0.90) than the estimate for 1993 (0.30). It seems that the large variation observed in 1993 was primarily caused by environmental factors, accounting for an increase of  $V_p$ , and hence a decrease of  $h^2$ . Considering the on average moderate heritability values, effective selection for total number of flowers seems possible. Particularly in the early generations, even mass selection will most likely be

Table 5.3. Heritability estimates ( $\pm$  s.e.) for flowering characteristics from parent-offspring regression as well as from variance components.

Parameter Method	1993	1994	1993 + 1994
Onset of flowering (d)			
Parent-offspring	0.90 $\pm$ 0.24	0.75 $\pm$ 0.16	0.82 $\pm$ 0.18
Variance components	1.50 $\pm$ 0.37	0.69 $\pm$ 0.29	0.94 $\pm$ 0.33
Slope, <i>B</i>			
Parent-offspring	0.04 $\pm$ 0.18	0.30 $\pm$ 0.22	0.17 $\pm$ 0.17
Variance components	0.16 $\pm$ 0.18	0.55 $\pm$ 0.27	0.34 $\pm$ 0.22
Peak bloom, <i>M</i> (d)			
Parent-offspring	0.87 $\pm$ 0.20	0.83 $\pm$ 0.15	0.85 $\pm$ 0.14
Variance components	1.11 $\pm$ 0.35	0.74 $\pm$ 0.30	0.68 $\pm$ 0.28
Number of flowers, <i>C'</i>			
Parent-offspring	0.51 $\pm$ 0.16	0.52 $\pm$ 0.23	0.51 $\pm$ 0.15
Variance components	0.30 $\pm$ 0.22	0.90 $\pm$ 0.32	0.29 $\pm$ 0.21
Duration of flowering (d)			
Parent-offspring	0.32 $\pm$ 0.17	0.43 $\pm$ 0.20	0.38 $\pm$ 0.16
Variance components	0.25 $\pm$ 0.20	0.45 $\pm$ 0.24	0.34 $\pm$ 0.22
Duration of flowering ( $^{\circ}$ Cd)			
Parent-offspring	0.29 $\pm$ 0.17	0.50 $\pm$ 0.20	0.39 $\pm$ 0.16
Variance components	0.27 $\pm$ 0.20	0.48 $\pm$ 0.25	0.44 $\pm$ 0.24

effective, provided that the effective population size is kept large enough to maintain ample genetic variation, and the environment allows for expression of genetic variation.

For duration of flowering (both expressed in chronological and thermal time) for 1994 moderate heritabilities were found with both estimation methods. However, for 1993, the heritabilities were rather low, and not significantly different from zero. As seemed to be the case for total number of flowers, the variation for duration of flowering in 1993 may have been increased due to environmental factors, or the variation in 1994 was decreased (i.e., because of early maturation). In populations with at least similar genetic variation for this trait, progress may be expected from mass selection, however, more demanding methods such as family selection could be considered as well.

The heritabilities for the slope parameter were all very low and not different from zero, except for the estimation based on variance components in 1994, which was somewhat higher. In general, this parameter does not seem to be suitable for selection.

### Correlations

Phenotypic correlations were only found between onset of flowering and date of peak bloom; the correlation coefficients were 0.93 and 0.82 for 1993 and 1994, respectively (Tables 5.4 and 5.5). This was expected since both parameters are correlated by definition (see equation 3). Also a significant additive genetic correlation was found between these traits (0.95 and 0.89 for



Table 5.4. Phenotypic and genetic correlations between flowering characteristics, based on the HS-family means of 1993.

Parameter					
Onset of flowering (d)	1				
Date of peak bloom, <i>M</i> (d)	2	0.93 <sup>1)</sup> <i>0.95<sup>1)</sup></i>			
Total number of flowers, <i>C'</i>	3	0.22 <i>0.43</i>	0.25 <i>0.43</i>		
Duration of flowering (d)	4	0.23 <i>0.57</i>	0.55 <i>0.80</i>	-0.03 <i>0.30</i>	
Duration of flowering (°Cd)	5	0.04 <i>0.49</i>	0.38 <i>0.74</i>	-0.01 <i>0.35</i>	- -
		1	2	3	4

<sup>1)</sup> Phenotypic correlations are printed in normal font, genetic correlations in *italics*.

Table 5.5. Phenotypic and genetic correlations between flowering characteristics, based on the HS-family means of 1994.

Parameter					
Onset of flowering (d)	1				
Peak bloom, <i>M</i> (d)	2	0.82 <sup>1)</sup> <i>0.89<sup>1)</sup></i>			
Total number of flowers, <i>C'</i>	3	0.36 <i>0.50</i>	0.18 <i>0.29</i>		
Duration of flowering (d)	4	-0.21 <i>0.27</i>	0.39 <i>0.69</i>	-0.32 <i>-0.13</i>	
Duration of flowering (°Cd)	5	0.01 <i>0.29</i>	0.58 <i>0.72</i>	-0.24 <i>-0.11</i>	- -
		1	2	3	4

<sup>1)</sup> Phenotypic correlations are printed in normal font, genetic correlations in *italics*.

1993 and 1994, respectively). Genetic correlations were also found between duration of flowering and date of peak bloom. For 1993 and 1994, values of respectively 0.80 and 0.69 were found when duration of flowering was expressed as number of days, and 0.74 and 0.72 when expressed in thermal time. The phenotypic correlations between those traits were well below 0.60, and considered not meaningful. In general, phenotypic correlations were found to be lower than genetic correlations. If the following relationship between phenotypic, genetic and environmental coefficients of correlation applies (Bos and Caligari 1995):

$$\rho_p = \rho_g h_x h_y + \rho_e e_x e_y \quad (\text{where } h = \frac{\sigma_g}{\sigma_p}, \text{ and } e = \frac{\sigma_e}{\sigma_p}, \text{ of traits } x \text{ and } y),$$

this could be explained if the environmental component ' $\rho_{e_x e_y}$ ' was negative. The environmental variance for some trait may differ from genotype to genotype, and likewise, the environmental correlation of two traits may vary across genotypes. The phenotypic correlation depends on both the genetic and environmental correlation, and particularly in genetically heterogeneous populations these may have very different values, even values of opposite signs (Bos and Caligari 1995).

Probably more interesting than the presented correlations, is the absence of particular correlations. The total number of flowers was not correlated with duration of flowering, which means that plants producing more flowers do not necessarily flower longer. Furthermore, onset of flowering was neither correlated with total number of flowers nor with duration of flowering. When characters are uncorrelated, selection for one these characters will, with sufficient sample size, not affect the variation of the other.

#### *Perspectives of breeding for flowering synchronisation*

The flowering model presented in this paper, describes the flowering of single plants of *D. pluvialis* very well. Useful selection parameters could easily be derived and compared between plants. Onset of flowering showed high heritability values, both from parent-offspring regression and from variance components. Particularly at high selection intensities, only a few generations of mass selection for this character will result in a population with a good flowering synchronisation between plants. The synchronisation within plants, i.e. duration of flowering, showed moderate to low heritabilities, but ample variation, suggesting that selection most likely will lead to improvement.

However, collection of the data necessary for determination of the curve-parameters is very laborious and impractical for large breeding programmes. In practice, onset of flowering could therefore probably be best defined as the date at which at least two open flowers are visible. Duration of flowering should then analogously be defined as the time span between the first two and the last two open flowers of a plant. This may be a useful definition when there is still much (genetic) variation. However, since flowering development was particularly irregular at start and ending of flowering, this approximation may be too rough in later stages of selection. The genetic correlation between duration of flowering and date of peak bloom (but not onset of flowering) suggests that maybe selection against plants with a late peak bloom would result in some reduction of duration of flowering. If further genetic improvement is required, a selection method should be chosen in which the number of plants to be screened for duration of flowering is small enough to apply the flowering model. The model may also be used in the search for other (genetically) correlated characters, or to examine treatment effects on duration of flowering.

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## Chapter 6

# Response to divergent mass selection for plant architecture and earliness and its effect on seed yield in *Dimorphotheca pluvialis*

### Summary

Seeds of *Dimorphotheca pluvialis* (L.) Moench contain oil with a unique fatty acid composition, making it a potential raw material for several industrial applications. So far, the species has not been used in arable farming. The currently available plant populations show considerable variation for morphological and agronomic traits. Divergent mass selection for plant architecture and earliness was performed, aiming at the creation of more uniform populations and subsequently the determination of the plant ideotype. Three cycles of combined selection for plant architecture and earliness resulted in a significant response for both traits in both directions, even at a low selection pressure. Selection for early flowering or procumbent plant architecture showed a better response and a higher realised heritability than selection for late flowering or upright plant architecture. At low plant densities (4 plants/m<sup>2</sup>), as used during selection, differences in plant architecture between procumbent and upright selections were clearly visible. However, at densities used in cultivation practice (50 plants/m<sup>2</sup>), the crops produced by the two plant types looked remarkably similar; between the plots no clear differences in plant architecture could be seen. The different plant architecture selections showed similar flowering development and seed yield, and therefore no ideal plant architecture type could be defined with regard to breeding for increased seed yield. Earliness, however, had a significant effect on seed yield; early flowering selections showed the highest yields. Since genotype by year interactions could not be determined, no firm conclusions on the ideotype with regard to earliness should be drawn. Nevertheless, (very) late flowering selections in general are undesired in the Netherlands because of an increased risk of experiencing unfavourable weather conditions during flowering and seed set.

### Introduction

In recent years, there has been much interest in developing new oilseed crops, which can be used for industrial purposes (Rexen 1992, Van Soest 1994). Particularly vegetable oils containing fatty acids with long carbon chains (>20), unusual desaturation positions and/or functional groups (e.g. hydroxy- and epoxygroups) are of interest for industry (Kleiman 1990, Princen and Rothfus 1984, Pryde 1979). One of the plant species having received considerable attention in the Netherlands is *Dimorphotheca pluvialis* (L.) Moench (Van Soest and Mulder, 1993; Van Soest, 1994). Seed oil from the genus *Dimorphotheca* (*Compositae*) contains large amounts (ca 60-65%) of dimorphecolic acid ( $\Delta^9$ -hydroxy,10t,12t-octadecadienoic acid). The highly reactive conjugated hydroxydiene structure of this fatty acid suggests that (after chemical modification) it is a versatile raw material in specialty applications, e.g. flavours and fragrances, pharmaceuticals, lubricants, plastic foams, coatings and nylons (Derksen *et al.* 1993, Knowles *et al.* 1965; Muuse *et al.* 1992). Studies have shown that the fatty acid can also be used in the industrial production of pheromones (J.T.P. Derksen, pers. comm.).

*D. pluvialis* is native to southern Africa, particularly the Cape region, where it is a common wild plant. Individual plants show considerable variation in plant architecture, ranging from compact and erect to open with procumbent branches. Depending on architecture, plant height is 10-50 cm. In general, plants have one main stem, with branches mainly at the base. When plants are widely spaced, particularly the basal branches are much ramified. Although environmental conditions influence growth habit, the variation for plant architecture in *D. pluvialis* is undoubtedly partially due to genotypical differences (Norlindh 1943). The flower heads are very conspicuous with yellow or purple disk florets and ray florets with one large white petal which is often purple at the base. The two types of florets produce distinct types of seed. Seeds produced by the disk florets are smooth and flattened and have two large 'wings', while those produced by the ray florets are more or less rod-shaped with a wrinkled surface (Barclay and Earle 1965, Norlindh 1943 and 1977). Both types of seed contain oil in similar quantities and of equivalent fatty acid composition (Earle *et al.* 1964). Besides 60-65% dimorphecolic acid, the oil contains 10-20% oleic acid, 10-14% linoleic acid, 2-3% palmitic acid, 1-2% stearic acid, and small amounts (<1.5%) of other fatty acids (Binder *et al.* 1964, Muuse *et al.* 1992 and 1994).

Though *D. pluvialis* is known in Europe since the 17th century as a garden ornamental (Eliovson 1973, Norlindh 1943), it has not previously been used as an arable crop for oil production. Evaluation experiments have shown that the species readily grows in the USA and Western Europe. In these areas, the species, being only moderately tolerant of frost, is grown best as a summer annual. When sown in April, it flowers in July and can be harvested in August. However, the reported seed yields were variable and rather low (400-900 kg/ha), mainly as a result of a long, unsynchronised flowering and ripening period combined with a poor seed retention (Knowles *et al.* 1965, Meier zu Beerentrup and Röbbelen 1987, Van Dijk and Borm 1993). Previous studies showed considerable variation between plants for synchronisation of flowering, both between and within plants (Chapter 5).

In modern farming, uniform plant populations are required. To reduce the variation for onset of flowering and plant architecture, (divergent) selection for those traits was performed. Realised heritability and response to selection were used to determine breeding strategies for both characteristics. Though intuitively an erect, compact plant architecture seems preferable over procumbent plant types in *D. pluvialis* (Willingham and White 1973), no studies on this subject have been reported. Therefore, also the effect of plant architecture and earliness on seed yield was studied.

## Materials and Methods

### *Plant material and selection procedures*

Four populations of *D. pluvialis* were used as base material from which plants were selected. The populations had been tested earlier in evaluation trials, and together these four populations were presumed to represent as

much variation for agronomic and morphological characteristics as possible. All populations originated from botanical gardens in Europe: population 879234 from Berlin (Germany), population 879729 from Århus (Denmark), population 879528 from Dijon (France) and population 879372 from Bonn (Germany). For reasons of readability, these base populations will be referred to as BP1, BP2, BP3 and BP4, respectively. In 1992, at the CPRO-DLO experimental farm in Lelystad, 400 plants of each of the base populations were grown on separate plots, at a density of 4 plants/m<sup>2</sup>, allowing scoring of individual plants.

In previous experiments, considerable variation between individual plants for onset of flowering was found (Chapter 5). In order to reduce undesired assortative mating resulting from variation for earliness within the selection populations, it therefore was decided to combine selection for plant architecture with selection for onset of flowering (earliness). Hence, six selection groups were distinguished: all possible combinations of two plant architecture types with three earliness classes. For plant architecture, selection was performed towards the two extreme types: procumbent (P) and upright (U). A schematic representation of these plant types is given in Figure 6.1. The term 'upright' was preferred over 'erect' to avoid confusion with the abbreviation 'E' for early flowering. For earliness, three classes were distinguished: early (E), middle (M) and late (L) flowering.

In the first selection cycle, from the four base population plots described above, approximately 25 plants were selected for each of the six selection groups. Of each selected plant, ten mature seed heads were harvested, and per group the seeds of the 25 selected plants were bulked. These six bulk selections were used as initial material for the next selection cycle. In the second and third selection cycle, the selection procedure was based on the method of 'independent culling levels'. Instead of fixed phenotypic levels, fixed proportions of selected plants were used. For earliness 33.3%, and next for architecture 25% of the plants were selected. The selection pressure was deliberately kept low, in order to maintain reasonable effective population sizes, and to limit the distance between the selected plants to enhance cross pollination. For the second selection cycle, six plots were sown (one per selection group), each consisting of 600 plants at a density of 4 plants/m<sup>2</sup>. Selection took place by first selecting the 200 plants meeting the criteria for earliness. For the 'early' and 'late' selections, of the respective plots the 200 earliest or latest flowering plants were selected, respectively, and the remainder removed. For the 'middle' flowering selections the 200 earliest flowering plants were removed, the next 200 flowering plants selected and the remainder removed. After selection for earliness, from the 200 remaining plants on each plot, 50 plants were selected, which best represented the desired architecture type. All other plants were removed from the field. From the remaining plants, immediately after selection open flowers and seed heads were removed, to ensure pollination among selected plants only. At maturity, ten seed heads per plant were harvested, and seed was bulked per selection group. These bulk selections were used as initial material for the third selection cycle, which was carried out the following year, using the same procedure as described for the second cycle. Again, after selection, ten seed

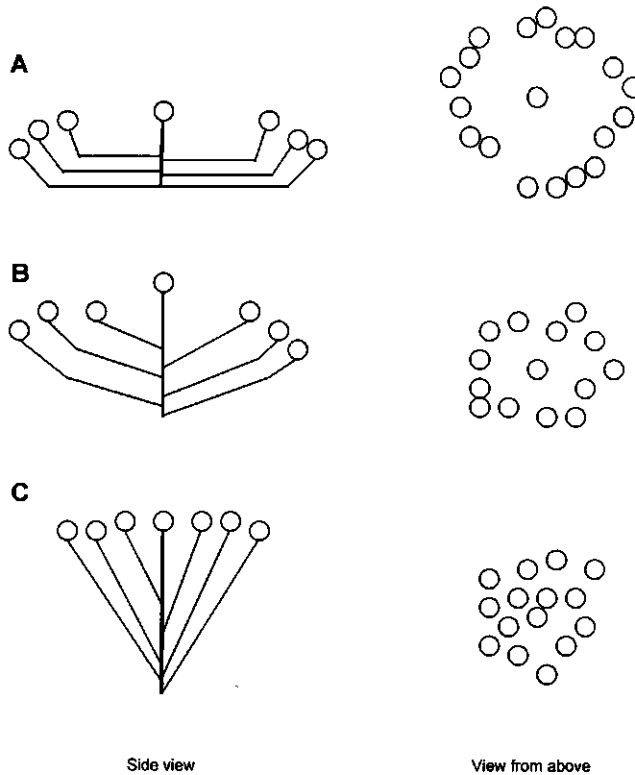


Figure 6.1. Schematic representation of plant architecture types in *Dimorphotheca pluvialis*. A = procumbent (score 1), B = intermediate (score 5) and C = upright (score 9). Semi-procumbent and semi-upright plant types (scores 3 and 7) not shown. For a full description of the plant types, see text.

heads per plant were collected at maturity, and per selection group the seed samples were bulked. All selections will be referred to with a code consisting of a combination of the earliness class and architecture type, followed by a number representing the selection cycle, e.g. EU1, MU2 or LP3.

#### *Response to selection*

To determine the effect of selection on plant architecture and earliness, all 18 selections (six groups, three selection cycles) as well as the four base populations were evaluated in one trial. All 22 objects were sown in a randomised complete block design with three replications. Each block consisted of 22 rows of 18 plants, to which the objects were randomly allocated. Distance between and within rows was 0.75 m, allowing scoring of individual plants.

To determine plant architecture, five plant types were distinguished (Figure 6.1):

- Procumbent plants (score 1) are characterised by a short, erect main stem and horizontally orientated branches, most of which are lying prostrate on the soil surface with only the apices ascending. Viewed from above, the plants generally show one central flower (of the main stem), surrounded at a distance by a circle of flowers of the branches.
- Semi-procumbent plants (score 3) have prostrate basal branches, but the apices generally start ascending closer to the main stem as compared to the procumbent plants. Also second and higher order branches are more or less ascending. Viewed from above, the central flower of the main stem can clearly be distinguished from a broad ring of flowers of the branches.
- Intermediate plants (score 5) have slightly ascending primary branches, orientated in a wide angle from the main stem. Branches in general rarely touch the soil surface. Viewed from above, the plant has an open structure, with the flower of the main stem still spatially separated from the other flowers.
- Semi-upright plants (score 7) have a relatively long main stem with more or less ascending primary branches. This plant type has a compact, globe-shaped appearance, with flowers on the outside. Viewed from above, there is no spatial separation between the flower of the main stem and those of other branches.
- Plants of the upright type (score 9) have a relatively long main stem with clearly ascending branches. Most flowers are situated in one plane, at the same height as the apical flower of the main stem. Viewed from above, the flower of the main stem can not be distinguished from the flowers of the branches.

The plant architecture of each individual was scored twice; first when the earliest plants started flowering, and a second time two weeks later when the majority of the plants had started flowering. The analyses were based on the mean of the two scores. Onset of flowering of plants (earliness) was defined as the number of days between sowing and opening of its fifth flower, and was determined weekly.

Differences between objects for plant architecture and earliness were tested using Genstat ANOVA procedures (Genstat 5 Committee 1989). The response to selection for plant architecture and earliness was determined per selection cycle by calculating the difference between the respective group means. Since in the first selection cycle plants were selected from four populations, the selection response of the first selection cycle was not determined. Realised heritabilities of the characteristics were calculated from

$$h^2 = \frac{R_{cum}}{S_{cum}},$$

where  $R_{cum}$  represents the total response, and  $S_{cum}$  the selection differential cumulated over all selection cycles (Falconer 1989). The selection differential per cycle could not be determined directly in the year of selection, since non-selected plants were removed from the field. Therefore, the selection differentials ( $S$ ) were estimated from the evaluation trial, by determining the respective generation mean ( $M$ ), and subtracting the mean of the plants that



would have been selected from these selection populations, if selection had taken place ( $M_s$ ):

$$S = M - M_s.$$

#### *Effects of selection on seed yield*

To assess the influence of plant architecture and earliness on seed yield, all 22 objects were also tested on 3 x 6 m field plots with a row distance of 25 cm and an aimed density of 50 plants/m<sup>2</sup>. The experimental layout was a randomised complete block design with three replications. To evaluate the effect of selection for earliness under normal plant density conditions, of each plot the number of flowers was counted in a 0.375 m<sup>2</sup> subplot at weekly intervals during a period of five weeks. Plots were harvested when most of the seed heads were mature, by cutting the whole crop. To avoid possible influences of adjacent plots, only the middle 1.5 x 6 m of each plot was harvested. The actual plant density was determined immediately after harvest by counting the stubbles. After drying, the crop was threshed and seed yield of both seed types, as well as their respective thousand seed weights, were determined. Data on yield components were analysed with ANOVA (Genstat 5 Committee 1989) in accordance with the experimental layout.

## Results and discussion

### *Response to selection for plant architecture and earliness*

The correlation between plant architecture and earliness (based on all individual plants of the evaluation trial) was very low:  $r^2 = 0.15$ . Selection for one trait has therefore probably not affected selection for the other.

The described scoring method with five discrete classes for the characterisation of the plant architecture was satisfactory. Of all plants scored

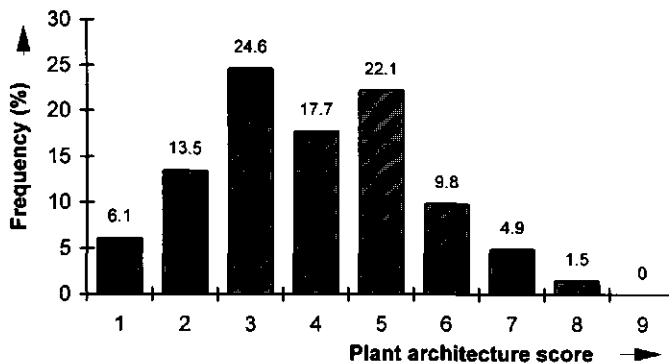


Figure 6.2. Frequency distribution of mean scores for plant architecture in the evaluation trial with individual plants (100% = 1102 plants).

Table 6.1. Population means and standard deviations for plant architecture and earliness of the base populations, based on the evaluation trial with individual plants.

Population	Plant architecture <sup>1)</sup>		Earliness <sup>2)</sup>	
	Mean	Stand. dev.	Mean	Stand. dev.
BP1	3.8	1.4	89.2	4.6
BP2	3.2	1.2	88.5	4.4
BP3	3.7	1.5	94.2	3.9
BP4	5.6	1.1	92.0	4.1
LSD <sub>0.05</sub>	0.5		1.6	

<sup>1)</sup> Score; 1 (procumbent) - 9 (erect). For a detailed description of plant architecture scores, see text.

<sup>2)</sup> Onset of flowering, expressed as days from sowing until five open flowers

in the evaluation experiment, 55% received the same score both times, and for 42% the second score was one class higher or lower than the first score. Of the latter group, the plant architecture was most likely intermediate between both classes, and therefore described best by the average of both scores. It was remarkable that none of the plants got twice score 9, while 6.1% of the plants received twice score 1 (Figure 6.2). Apparently, in the used populations/selections true procumbent plants are not uncommon and true upright plants are rare. Indeed, the average scores of base populations BP1, BP2 and BP3 were rather low, only few plants were of the upright type (Table 6.1). Population BP4 had a higher average score of 5.6, in this population less procumbent, and relatively more upright plants were found.

The differences in plant architecture between the selections were highly significant ( $P \leq 0.001$ , Table 6.2). From comparison between the selections of the third and the first cycle, it could be seen that progress had been made for

Table 6.2. Selection means (with standard deviations in brackets) for plant architecture<sup>1)</sup>, and response to selection, based on the evaluation trial with individual plants.

Selection		Cycle		
		1	2	3
Upright	Early	4.6 (1.4)	4.7 (1.5)	4.9 (1.4)
	Middle	4.2 (1.4)	4.5 (1.2)	5.5 (1.4)
	Late	4.5 (1.4)	4.5 (1.4)	5.1 (1.2)
	Mean	4.5	4.6	5.2
	Response	-	0.1	0.6
Procumbent	Early	4.2 (1.2)	2.5 (1.0)	2.4 (1.2)
	Middle	3.8 (1.5)	2.9 (1.1)	2.6 (1.0)
	Late	3.7 (1.6)	3.1 (1.3)	2.7 (1.3)
	Mean	3.9	2.8	2.6
	Response	-	1.1	0.2

LSD<sub>0.05</sub> = 0.5

<sup>1)</sup> Score: 1 (procumbent) - 9 (erect). For a detailed description of plant architecture scores, see text.

both plant architecture types. Although within architecture types the response of the earliness classes differed in magnitude, the trend was similar. For the upright plant type, progress was made particularly in the third selection cycle, and

Table 6.3. Realised heritabilities for plant architecture after three cycles of combined selection for plant architecture and earliness.

Earliness	Architecture	
	Upright	Procumbent
Early	0.07	0.67
Middle	0.47	0.38
Late	0.18	0.30
Pooled over earliness	0.23	0.44

for procumbent plants in the second selection cycle. For procumbent plants the overall response was much larger than for upright plants. Furthermore, the most advanced upright selection (MU3) was not better than BP4, while the most advanced procumbent selections scored significantly lower (more procumbent) than the four base populations. Therefore, with equal selection pressure, selection for procumbent plants was apparently more effective than selection for upright plants. The difference in response to selection for both architecture types was reflected in the realised heritabilities. Pooled over earliness classes, the realised heritabilities for upright and procumbent plant types were 0.23 and 0.44, respectively (Table 6.3).

Though divergent selection for plant architecture was effective, selection resulted in only a slight reduction in the variation within the selection populations, as can be seen from the respective standard deviations (Table 6.2). To improve uniformity of the populations, continuation of selection is required, preferably with a higher selection pressure.

As far as selection for earliness was concerned, the response to selection for earlier flowering plants was only significant in the second cycle, while the response to selection for later flowering plants was only significant in the third

Table 6.4. Selection means (with standard deviations in brackets) for earliness (days from sowing until five open flowers), and response to selection, based on the evaluation trial with individual plants.

Selection		Cycle		
		1	2	3
Early	Upright	87.4 (4.1)	85.4 (3.9)	84.6 (3.9)
	Procumbent	88.6 (3.9)	84.3 (3.9)	83.4 (3.1)
	Mean	88.0	84.9	84.0
	Response	-	3.1	0.9
Middle	Upright	87.0 (4.8)	87.6 (5.1)	90.7 (3.6)
	Procumbent	90.7 (4.8)	88.3 (4.7)	88.8 (4.0)
	Mean	88.9	88.0	89.8
Late	Upright	91.3 (4.1)	90.3 (3.8)	91.9 (4.6)
	Procumbent	88.4 (3.8)	89.5 (4.5)	92.8 (4.8)
	Mean	89.9	89.9	92.4
	Response	-	0.0	2.5

LSD<sub>0.05</sub> = 1.6

Table 6.5. Realised heritabilities for earliness after three cycles of combined selection for plant architecture and earliness.

Architecture	Earliness	
	Early	Late
Upright	0.37	0.07
Procumbent	0.78	0.72
Pooled over architecture	0.56	0.34

selection cycle (Table 6.4). The difference between the responses of the second and third selection cycle might be explained by differences in weather conditions during selection. In general, very late flowering plants start

flowering late August, early September, a time at which the weather conditions in the Netherlands are usually unstable. Cycle 2 was carried out in 1993, a year with dark, cold and wet weather during July, August and September. In that year, late genotypes may have had a selective disadvantage due to unfavourable weather conditions during seed set, possibly leading to seed with inferior quality. Cycle 3 was carried out in 1994, when weather conditions in July and August were very warm and dry, accelerating flowering and to some extent complicating selection of the earliest plants. Late flowering plants still experienced relatively good weather conditions during flowering, allowing a good seed set and thus a 'normal' contribution to the next generation. The overall response for early flowering after three cycles of selection was 4.0 days, and for late flowering 2.5 days. The earliest selections (EU3 and EP3) flowered significantly earlier than the earliest base population (BP2), but the latest selections (LU3 and LP3) did not exceed the latest base population (BP3). In this experiment, selection for early flowering was more effective than for late flowering. Realised heritabilities for earliness, pooled over architecture types were 0.56 and 0.34 for early and late selections, respectively (Table 6.5). These values are somewhat lower than heritability estimates for onset of flowering from other experiments with *D. pluvialis* (Chapter 5), but in those experiments other populations and estimation methods were used.

In the early and middle selections, selection led to more uniform flowering populations, as can be seen from the standard deviations of the respective populations (Table 6.4). For the late selections however, the variation of the cycle 3 selections was even larger than that of the corresponding selections of cycles 1 and 2. For improvement of uniformity, i.e. synchronisation of flowering, continued selection, particularly with a higher selection pressure, is necessary. At the same time, the observed variation in the cycle 3 selections enables selection for still earlier or later flowering populations.

#### *Effects of selection on seed yield*

In the previous section it was established that selection for plant architecture and earliness was effective when plants were grown at a low density, with virtually no competition between neighbouring plants. The effect of earliness and plant architecture on flowering and yield under 'normal' field conditions, i.e. at a density of 40-60 plants/m<sup>2</sup>, was examined by sowing the same base populations and selections in small field plots, approaching the cultivation practice as much as possible.

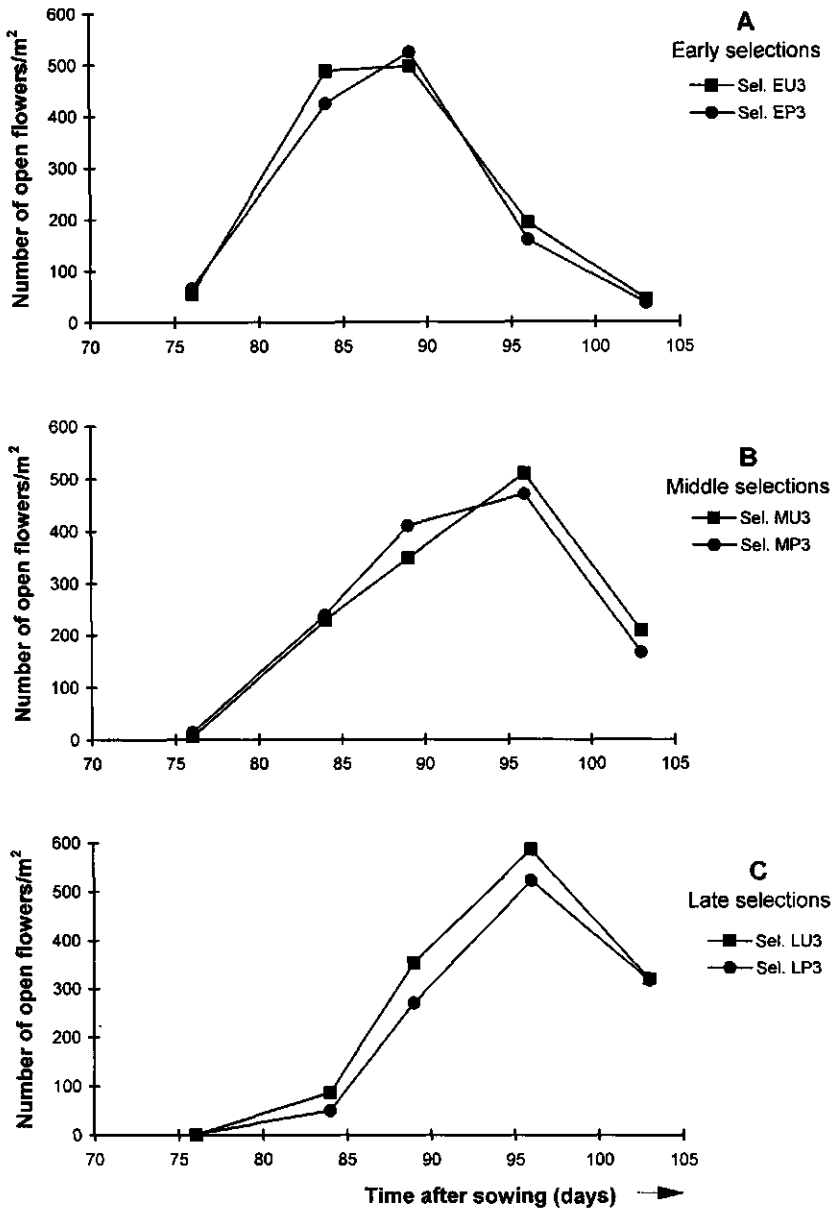


Figure 6.3. Course of flowering of selections obtained after three cycles of combined selection for earliness and plant architecture, measured on field plots. Figures A, B and C represent early, middle and late flowering selections, respectively. Within each figure upright (square markers) as well as procumbent (round markers) selections are presented.

Plant establishment in general was satisfactory, although BP3, BP4 and the middle and late selection populations of cycle 1 had a significantly lower density than 50 plants/m<sup>2</sup>. The reason for this is not quite clear, but it is possible that the longer seed storage period of the base populations and the cycle 1 selections may have negatively influenced seed viability.

Unfortunately, at these high plant densities, plant architecture at onset of flowering could not be determined, since individual plants could not be distinguished. Plots of upright and procumbent plant selections looked remarkably alike at onset of flowering, at these high densities genetic differences in plant architecture did not show. Probably the high plant density caused competition for space between plants, and resulted in a more ascending growth of the branches of procumbent plant types.

The effect of selection for earliness was clearly visible. The course of flowering of the early, middle and late selections of the third cycle is presented in Figures 6.3A, B and C, respectively. Within each graph, the upright and procumbent selections behave similarly. However, comparison between graphs shows that onset and peak of flowering of early selections are indeed earlier than those of middle and late selections, and that particularly onset of flowering of late selections is later than that of early and middle selections.

The yield components of the four base populations are presented in Table 6.6. Population BP3 had the lowest total seed yield of 42 g/m<sup>2</sup>. Population BP2 performed significantly better with a total seed yield of 71 g/m<sup>2</sup>. Thousand seed weight of rods and wings were approximately 2.0 and 1.8 g, respectively, and differences between base populations for this trait were not significant.

To examine the effect of selection for plant architecture on yield, data were pooled over earliness classes. No significant differences were found between the selections for yield of winged seeds, total seed yield and thousand seed weight of both seed types, despite some differences in plant density (Table 6.7). Apparently, plants of *D. pluvialis* are able to compensate for space, and plant architecture does not affect seed yield when populations are planted at 'normal' densities.

The effect of earliness on seed yield was examined analogously by pooling data over plant architecture types. Significant differences were found for all yield components (Table 6.8). The highest seed yields were found in the early selections, and the lowest yields in the late selections. The weather conditions during plant development, pollination and seed set most likely were different for early and late selections, particularly because the difference in earliness was fairly large in this experiment. Since the experiment was not repeated, genotype by environment and, more specifically, genotype by year interactions could not be determined, and no firm conclusions on the ideotype with regard to earliness should be drawn. However, in general, in the Netherlands (very) late flowering plants are undesired, because of an increased risk of experiencing unfavourable weather conditions during flowering and seed ripening.

Table 6.6. Yield components of the base populations (field plots).

Population	Density (plants/m <sup>2</sup> )	Seed yield (g/m <sup>2</sup> )			1000 seed weight (g)	
		rods	wings	total	rods	wings
BP1	43.6	24.3	40.0	64.2	2.17	1.89
BP2	55.6	27.4	43.6	70.9	2.21	1.81
BP3	37.3	18.4	23.5	41.9	1.79	1.75
BP4	38.6	17.0	33.7	50.7	1.85	1.71
LSD <sub>(0.05)</sub>	10.3	5.6	11.7	14.8	ns <sup>1)</sup>	ns <sup>1)</sup>

<sup>1)</sup> ns = no significant differences (P=0.05)

Table 6.7. Yield components of the selections pooled over earliness classes (field plots)

Architecture class	Selection cycle	Density (plants/m <sup>2</sup> )	Seed yield (g/m <sup>2</sup> )			1000 seed weight (g)	
			rods	wings	total	rods	wings
Upright	1	37.8	21.7	38.5	60.2	1.97	1.77
	2	55.7	23.8	38.2	62.0	1.95	1.71
	3	51.9	25.5	36.6	62.1	2.04	1.75
Procumbent	1	39.9	23.4	39.1	62.5	1.92	1.76
	2	52.9	23.8	37.0	60.8	1.95	1.72
	3	45.2	23.3	37.4	60.7	1.97	1.74
LSD <sub>(0.05)</sub>		6.6	3.7	ns <sup>1)</sup>	ns <sup>1)</sup>	ns <sup>1)</sup>	ns <sup>1)</sup>

<sup>1)</sup> ns = no significant differences (P=0.05)

Table 6.8. Yield components of the selections pooled over plant architecture classes (field plots)

Earliness class	Selection cycle	Density (plants/m <sup>2</sup> )	Seed yield (g/m <sup>2</sup> )			1000 seed weight (g)	
			rods	wings	total	rods	wings
Early	1	44.5	23.2	39.7	62.9	1.96	1.69
	2	50.4	28.5	46.7	75.2	2.15	1.74
	3	46.9	26.4	43.6	70.0	2.23	1.78
Middle	1	35.3	23.7	40.0	63.7	1.96	1.81
	2	55.1	22.8	40.9	63.7	1.86	1.75
	3	47.6	24.2	35.3	59.5	1.96	1.75
Late	1	36.7	20.9	36.6	57.5	1.91	1.79
	2	57.3	20.0	25.3	45.3	1.84	1.66
	3	51.1	22.7	32.0	54.7	1.83	1.70
LSD <sub>(0.05)</sub>		7.9	3.9	7.9	9.8	0.27	0.09

## Breeding perspectives

Both plant architecture and earliness readily respond to mass selection, even at low selection intensities. Continued (mass) selection, particularly at higher selection intensities, is expected to lead to a considerable increase in uniformity of selection populations. The observed variation within the present populations suggests that further divergent selection is possible. The generation of completely procumbent populations (score 1) as well as almost upright plant populations (score 8-9) seems feasible, if desired. For earliness, populations flowering as early as 70 to as late as 100 days after sowing can be established. Earliness and plant architecture type are not correlated, and selection for both traits can be performed independently. From this experiment no ideotype for plant architecture could be determined, since plant architecture apparently did not affect seed yield when plants were grown at densities used in cultivation practice. However, erect plant types may be preferable in general. Firm conclusions on the earliness ideotype could not be drawn from this experiment, but in the Netherlands, (very) late flowering populations have an increased risk of experiencing unfavourable weather conditions during flowering and seed set and should therefore be avoided.

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## Chapter 7

# **Current position and future perspective of *Dimorphotheca pluvialis* as an oilseed crop for industrial applications, with emphasis on agronomy and breeding**

## **Introduction**

Since the discovery of dimorphecolic acid in the seed oil of *Dimorphotheca pluvialis* (L.) Moench in 1960 (Earle *et al.* 1960, Smith *et al.* 1960), attention for this species has been varying. First, the unique hydroxydiene structure of the molecule raised the interest of many scientists in the USA, resulting in several optimistic publications on possible uses of dimorphecolic acid in industry (e.g. Diamond *et al.* 1964, Freedman *et al.* 1966, Knowles *et al.* 1965, Princen and Rothfus 1984, Pryde 1979). Despite these promising papers, the industrial interest for *D. pluvialis* slowly decreased, probably because of the rather poor agronomic performance of the species in evaluation trials in the USA (Barclay and Earle 1965, Willingham and White 1973). In Europe, the production of agricultural raw materials for industrial use regained interest in the 1980s. One of the first large screening programmes on industrial crops in Europe was carried out in Germany (Meier zu Beerentrup and Röbbelen 1987). In this programme, 53 plant species were evaluated for agronomic performance, including *D. pluvialis*. Not much later this species also featured in several international projects co-ordinated from within the Netherlands (see Chapter 1). At that time, several European industries acknowledged the versatile chemical potential of dimorphecolic acid and showed modest interest. Within the framework of the mentioned research programmes, particularly the first part of the production chain, from seed production to oil recovery, has been extensively investigated. In the next sections of this chapter, the most relevant results of these projects are summarised and discussed, in order to review the current prospects and constraints of *D. pluvialis* as an oilseed crop for industrial applications. In agreement with the scope of this thesis, particular attention is given to the prospects of crop improvement by means of breeding.

## **Agronomy**

*D. pluvialis* is only moderately tolerant of frost. In South Africa mature plants were reported to survive -15 °C (Eliovson 1973), but in the Netherlands young plants already died at temperatures around -9 °C (Wingelaar 1990). Cultivation in a frost-prone environment should therefore better be avoided. The species is daylength insensitive (Kreuzer *et al.* 1996). In its centre of origin in South Africa, where winters are relatively mild, it is often grown as a winter flowering garden ornamental (Eliovson 1973). There, it can be sown

early April and flowering starts mid winter, at the end of June. In southern Europe, seed can be sown in September and plants start flowering in spring. In the colder areas of north-west Europe, where winter frosts are common, *D. pluvialis* can only be grown as a summer annual. In this area the best results are achieved when the crop is sown early to mid-April (Meier zu Beerentrup and Röbbelen 1987, Rutgers 1991, Van Dijk en Borm 1994a, Wingelaar 1990). Plants emerge about two weeks later, in the course of April, when the risks of severe frost damage generally are reduced.

Sowing can be carried out with conventional sowing equipment normally used for cereals or legumes, preferably using the unwinged seeds. Seeds should be sown 1-1.5 cm deep, in rows ca 25 cm apart. Optimal plant density is 70-100 plants/m<sup>2</sup>, requiring a rate of ca 5-7 kg/ha of (unwinged) seeds. Higher plant densities do not increase seed yield. A wider row spacing (up to 50 cm) and lower plant densities (down to 50 plants/ m<sup>2</sup>) do not necessarily reduce seed yield but delay soil coverage of the crop, increasing possible weed problems (Van Dijk and Borm 1994a). The use of herbicides for weed control in new crops is subject to strict regulations. In research trials the best results were obtained with pendimethalin, propachlor and asulam, applied before seedling emergence. After emergence, mechanical weed control is preferred (Van Dijk and Borm 1994b).

*D. pluvialis* grows best on light, well-drained soils, e.g. sand or loamy clay. The crop does not require large amounts of fertiliser. In field trials, optimal yields were obtained after application of ca 100 kg N per ha, though the crop also grew satisfactory at rates of 40-80 kg N per ha (Van Dijk and Borm 1994b). This is in accordance with findings of Kreuzer *et al.* (1996), who found that a well fertilised and well established crop of *D. pluvialis* absorbed 100 kg N, 18 kg P and 140 kg K per ha during the growing season. Growth of the leaf area of the crop after seedling emergence progresses rather slow. The maximum leaf area index (LAI) of 3-3.5, is attained about nine weeks after seedling emergence. The LAI reaches its maximum shortly before start of flowering, and decreases during flowering and seed ripening. The total biomass production of a good crop is ca 6.500 kg/ha, including ca 1.600 kg seed. The highest reported harvest index was 28 (Wingelaar 1990), but more often realised harvest indexes ranged between 10 and 20, which is rather low compared to commercial seed crops (Kreuzer *et al.* 1996).

*D. pluvialis* is susceptible to a large number of pests and diseases. In the USA, particularly downy mildew, caused by *Plasmopara halstedii* (Farl.) Berl. & De Toni, has accounted for severe crop damage. In the field up to 60% of the plants were killed by this disease before seed ripening (Leppik 1965). Other reported pathogens include *Alternaria* spp., *Botrytis cinerea* Pers., *Cephalosporium* spp., *Fusarium* spp., *Puccinia flaveriae* Jacks., *Pythium ultimum* Trow., *Rhizoctonia solani* Kühn., *Sclerotinia sclerotiorum* (Lib.) De Bary, and *Verticillium* spp. (Leppik 1965, Willingham and White 1973). In the Netherlands, greenhouse and field trials showed that *D. pluvialis* is a good host to the soil-borne fungus *Rhizoctonia solani*, but a poor host to *Verticillium dahliae* Kleb. The species is also found to be susceptible to the root-knot nematodes *Meloidogyne hapla* Chitwood and *M. chitwoodi* Golden *et al.*, the

root-lesion nematode *Pratylenchus penetrans* Cobb., but not susceptible to the beet cyst nematode *Heterodera schachtii* Schm. (Kok *et al.* 1994, Verheul and Struik 1994). Aboveground, in Germany one attack of powdery mildew (not specified) was reported (Meier zu Beerentrup and Röbbelen 1987), and in the Netherlands incidentally plants with symptoms of rust (*Puccinia* spp.), downey mildew (*Bremia* spp.), and *Sclerotinia sclerotiorum* were found. For these diseases much variation in susceptibility between plants was found within the same trial fields, ranging from plants without any symptoms to plants completely wilted and perished (Chapter 5). The only disease which caused considerable damage to entire field crops of *D. pluvialis* in the Netherlands was *Botrytis cinerea*. Particularly in years with cool and wet weather conditions during flowering and seed ripening, flower heads were attacked by the fungus and seed yields were severely reduced (Mulder and De Bie 1994). In later experiments, often a fungicide was applied preventively during flowering (Breemhaar and Bouman 1995, Van Dijk and Borm 1994a).

Seed ripening of *D. pluvialis* in north-west Europe generally starts about three months after emergence, in mid-July. The optimal time of harvest is rather difficult to determine, but generally takes place early August. When harvested too early, yield losses occur due to incomplete filling and maturation of the seeds, which also negatively affects the quantity of the seed oil (Van Dijk and Borm 1993). Very immature seeds also have a reduced dimorphecolic acid content (Derksen *et al.* 1993). When harvested too late however, seed shattering will account for a severe reduction of seed yield. Even under 'optimal' harvest conditions, seed losses of 20%, entirely attributed to seed shattering, were observed (L. Hof, unpublished data). Direct combine-harvesting of the crop was not suitable because of the high moisture content of stems and foliage at time of harvest. Combine-harvesting after windrowing also proved to be unsuccessful, the windrows tended to be too compact and did not dry fast enough. With the currently available plant material, the most suitable method for harvesting large fields of *D. pluvialis* appears to be combining after chemical desiccation. Application of the desiccant, e.g. diquat (Reglone, 3 l/ha), should take place when 25-50% of the seeds is mature, and before severe seed shattering occurs. Circa 4-7 days later the crop can be combine-harvested (Breemhaar and Bouman 1995). This method resulted in a high amount (ca 80%) of harvested impurities, mainly consisting of pulverised plant material, immature flower heads, soil and dust. However, seeds could be cleaned satisfactory by using an air-screen cleaner, followed by a brushing machine and an indented cylinder separator (Breemhaar and Bouman 1995).

### Seed composition and oil recovery

The screening of large numbers of seed samples for oil content is inevitable when breeding for this trait, and requires a rapid, preferably non-destructive method. For winged seeds of *D. pluvialis*, a screening method based on Near Infrared Reflection Spectroscopy (NIRS), has been developed

(Groenendijk and Frankhuizen 1993). The use of low resolution pulse Nuclear Magnetic Resonance (NMR) was also investigated, but when measurements were performed at room temperature, the results for seeds of *D. pluvialis* were unreliable, apparently due to the high viscosity of the seed oil (Van der Kamp *et al.* 1992 and 1993).

Both types of seeds of *D. pluvialis* have a similar oil content and fatty acid composition (Earle *et al.* 1964, Meier zu Beerentrop and Röbbelen 1987). Seed oil content of individual plants used in the experiments described in this thesis ranged from 14-30% (L. Hof, unpublished data), exceeding the previously described range of 15-25% for this character (Barclay and Earle 1965, Derksen *et al.* 1993, Rutgers 1991). Besides 60-65% dimorphecolic acid, the oil contains 10-20% oleic acid, 10-14% linoleic acid, 2-3% palmitic acid, 1-2% stearic acid and small amounts (<1.5%) of other fatty acids (Binder *et al.* 1964, Muuse *et al.* 1992 and 1994). Oil quality is not only determined by oil composition within the seed; it may also be affected by the method of oil recovery (Muuse *et al.* 1994). Mechanical oil expelling of the seeds resulted in an oil with a high viscosity and a dark green colour, caused by unsaponifiable substances such as carotenes, chlorophyll and sediments. Furthermore, the efficiency of oil recovery by pressing, even at high temperatures, was very low, about 40%. Moreover, pressing at high temperatures resulted in degradation of the dimorphecolic acid. Oil recovery by means of supercritical carbon dioxide (CO<sub>2</sub>) extraction or organic solvent extraction was much more efficient, both methods yielded more than 95%. Oil of *D. pluvialis* obtained by extraction with organic solvents as hexane or pentane, had a dark green colour and needed further refining to remove pigments and gums, whereas oil obtained by supercritical carbon dioxide extraction was light yellow and needed no further refining. The latter oil also had a low phospholipid content (11 mg P/kg), while pressed or hexane- and pentane extracted oils had much higher phospholipid contents (200 mg P/kg). The dimorphecolic acid content of the oils recovered by the various techniques (except for pressing at high temperatures) showed only minor differences (Muuse *et al.* 1994). Apparently, supercritical carbon dioxide extraction seems the preferred method for recovery of the oil of *D. pluvialis*, although the processing costs of this method are substantially higher compared to the other described methods (Boswinkel *et al.* 1996). For isolation of the dimorphecolic acid from the oil, conventional high-pressure/high-temperature fat splitting processes are unsuitable, as dimorphecolic acid is thermo-labile. However, mild enzyme mediated hydrolysis of the oil of *D. pluvialis* with 1,3-positional specific lipases showed promising results. With this method the reactive conjugated hydroxylgroup of dimorphecolic acid remained intact. In a bioreactor system where the lipase was immobilised on a membrane, even a continuous production of dimorphecolic acid could be established (Derksen *et al.* 1993). Tassignon (1995) developed useful procedures for the production of derivatives of dimorphecolic acid, which can be used as building blocks for further synthesis. Both oil from seeds that had been in storage for more than one year as well stored oil, showed degradation due to hydrolytic deterioration (up to 50%) of the glycerides and dimerisation (up to 20%) of the hydroxy-

esters and -acids. (Tassignon 1995). Though even this 'inferior' oil is still valuable base material, oil extraction of fresh seeds is preferred.

As the seed contains only ca 20-25% oil, the rest fraction (seed meal) is considerable and an adequate utilisation of this by-product may contribute to an economically feasible production of the crop. Seed meal of traditional oil crops is often used as feed ingredient for livestock (Van Soest *et al.* 1991). For this purpose, protein content, protein composition, content of anti-nutritional factors and fibre content of the meal are important quality factors. Analyses showed that protein content of the seed meal of *D. pluvialis* is rather low (ca 21%) compared to e.g. meal of rapeseed (ca 32%) or soybean (ca 40%). As for protein composition, the content of amino acids as lysine, methionine and cystine is somewhat low for optimal growth of rodents, but otherwise it appears to be a suitable source of essential amino acids (Van Etten *et al.* 1961). In-vitro digestibility tests showed moderate values for ruminants and rather low values for pigs (Steg *et al.* 1994). No information has been found on the presence of anti-nutritional factors in seeds of *D. pluvialis*. The fraction of (fibrous) cell walls in the meal is ca 50%. Cell walls are composed mainly (ca 60%) of cellulose (Steg *et al.* 1994).

Oil and proteins are mainly situated in the embryo, which comprises only ca 40% of the whole seed (Knowles *et al.* 1965). Removal of the seed hull prior to oil recovery would therefore result in considerable improvement of feed quality of the residual meal. At present, there seem to be no major constraints for using seed meal of *D. pluvialis* as part of livestock diets.

### **Possibilities of crop improvement by means of breeding**

From the results described in the previous sections, several limitations of *D. pluvialis* as an arable crop have been identified. In this section, the possibilities of genetic improvement by means of breeding will be briefly discussed.

Physiological research revealed that the growth of the leaf area of the crop after seedling emergence is rather slow. The maximum leaf area index (LAI) did not exceed the value of 3.5 and was only maintained at this level for a rather short period of time, limiting total biomass production. Furthermore, the harvest index was found to be rather low. As was described in Chapter 6, much variation for both plant architecture and earliness was found. Moreover, these characters showed moderately high heritabilities, and mass selection for these characters was effective. Apart from the differences in architecture, also much variation for morphological characteristics as leaf size and shape, or number and position of branches was observed (data not shown). Variation for these characters was earlier observed by other authors, but not quantified (Chapter 1). In Chapter 5, substantial differences in total number of flowers per plant, earliness, and duration of flowering were described. It is therefore very likely that selection for an earlier soil cover, a higher LAI and even a higher harvest index is possible. Prognoses on the degree of improvement, however, would be rather speculative.

*D. pluvialis* has been described as being susceptible to many pests and diseases, although in cultivation practice in the Netherlands only few accounted for reduction in yield (see above). When dark and wet conditions occur during flowering and seed ripening, *Botrytis cinerea* may cause considerable damage, but this fungus can be controlled relatively easily by application of fungicides. For a healthy crop rotation, control of soil-borne diseases is probably most important. In general, *D. pluvialis* has been described as a host to several nematodes such as *Meloidogyne* spp. and *Pratylenchus penetrans*, and soil-borne fungi such as *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, but no data have been published on differences in resistance within or between populations. As *D. pluvialis* shows large variation for many characteristics, it is not to be excluded that the species also shows considerable variation in resistance to pests and diseases. For instance, in the field trials described in Chapters 3 to 6, plants showing severe symptoms of *Sclerotinia sclerotiorum* and *Puccinia* spp. grew close to plants showing no or only few symptoms (data not shown). Improvement of resistance to these diseases therefore seems very well possible, and for other pests and diseases variation in resistance within and between populations first needs to be assessed.

The most important limitation of an economically feasible seed production of *D. pluvialis* is probably the long, unsynchronised period of flowering and seed ripening, combined with the poor seed retention. The unsynchronised flowering and seed ripening hampers determination of the optimal time of harvest. Good seed retention could enable harvesting after completion of seed ripening. However, with poor seed retention as observed in *D. pluvialis*, severe yield losses are unavoidable. Improvement of synchronised flowering could partly be achieved by synchronisation of the onset of flowering. In Chapters 4 and 5 of this thesis, it was established that heritability estimates for onset of flowering were moderate to high, suggesting that quick progress could be expected from selection. This was confirmed by the results described in Chapter 6, where three cycles of selection led to a significant response, despite low selection intensities. However, as was elaborately discussed in Chapter 5, for a good synchronisation also duration of flowering needs to be taken into account. Heritability estimates for this character were moderate to rather low (Chapter 5). Progress from mass selection for this character may very well be expected, although somewhat more demanding methods such as family selection should be considered. Differences in seed retention between plants have been observed, but could not be quantified (unpublished results). It was also not possible to distinguish between genetic and environmental effects controlling this feature. As research on seed retention was not pursued in *D. pluvialis*, the possibilities of improvement by means of breeding are unclear. In many established seed crops however, the problem of seed shattering has been overcome by selection for improved retention. Further research on the mechanism of seed shedding, and the development of screening techniques detecting genetic differences in seed retention would greatly contribute to resolving this important problem in *D. pluvialis*.

The fatty acid composition of the seed oil seems to be rather constant, with a dimorphelic acid content ranging between 60 and 65%. Therefore, major changes in composition cannot be expected from selection. If such changes should be required, induced mutations or more advanced biotechnological techniques could be attempted. The seed oil content of *D. pluvialis* is as yet somewhat low, but substantial variation between plants has been found (Chapters 3 and 4). Moreover, the moderate heritability estimates for this character, combined with the observed variation, indicate that substantial improvement may be expected from selection. As was stated in Chapter 3, an average oil content of at least 30% will, most likely be feasible. In sunflower, rather spectacular increases in seed oil content from 25 to 45% were obtained by reduction of the seed hull fraction (Lühs and Friedt 1994). As dehulled seed of *Dimorphotheca* spp. also showed a considerably higher oil content (Knowles *et al.* 1965), it is likely that selection for decreased seed hull content will lead to a further increase of seed oil content. Furthermore, a lower seed hull content would benefit the quality of the seed meal for use as feed ingredient.

Particularly for breeding, knowledge of the mating system of the crops is essential. In the literature, *D. pluvialis* is described as a predominantly outcrossing species (Norlindh 1977). The experiments presented in Chapter 2, where exclusion of insects led to a severe decrease in seed set, support this observation. However, as limited seed set did occur in the absence of insects, it is possible that some self-fertilisation may have taken place. Glasshouse experiments confirmed that *D. pluvialis* is indeed capable of forming viable seeds after self-pollination (data not shown). Further research on details of the mating system of this species is highly recommended.

## Conclusions and future crop perspectives

*Dimorphotheca pluvialis* is as yet not ready for commercialisation. Although some research on agricultural performance was already carried out more than 30 years ago, the research on the possibilities of crop improvement by means of breeding was carried out in the framework of the VOICI, NOP and VOSFA projects (Chapter 1), and only started in the early 1990s. On the development scale as presented in the '4-ex' model described in Chapter 1, *D. pluvialis* can be considered to be positioned somewhere in the first half of the examination stage. The development of plant populations suitable for commercial seed oil production will take at least another 7-10 years. The present 'state-of-the-art' and future perspective of *D. pluvialis* as an oilseed crop for industrial applications are summarised below.

*D. pluvialis* is well adapted to the climatic conditions of north-west Europe, and fits well in a crop rotation system with annuals. Its susceptibility to soil-borne diseases should be taken into consideration, but so far has not caused major crop damage. Other pests and diseases seem to be easily controlled by agrochemicals, and improvement of disease resistance can most likely be achieved. Oil content of the seed and the synchronisation of flowering and



seed ripening of the crop have good prospects for improvement by means of breeding. Seed production might be increased by selection for genotypes with quick soil cover and improved harvest index. Substantial improvement of seed retention is essential for an economically feasible production of seed oil. As this problem has been overcome in many other established seed crops, it is well worth pursuing research on this topic in *D. pluvialis*. The oil can be recovered from the seeds satisfactorily, albeit with relatively expensive methods. At present, oil of *D. pluvialis* will be particularly suitable for use in products with a high added value (Tassignon 1995). Only one end-use of dimorphelic acid, the production of pheromones, has been developed so far, though it has not been commercialised. However, many of the potential applications described earlier have not been explored yet; therefore industry may well regain interest in the crop in the (near) future. The unique structure and functionalities of dimorphelic acid justify further research.

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## Summary

World-wide, but particularly in Western Europe and the USA, the interest in arable crops for non-food use has increased substantially over the past few decades. Surpluses of the major food crops and the industrial interest for renewable resources have led to research and development programmes aiming at the introduction of crops with industrial applications. Particularly vegetable oils with fatty acids containing functional groups are very attractive as substitutes for mineral oils used in the production of e.g. lubricants, surfactants, coatings and polymers. Therefore, in recent years much effort has been made to domesticate wild species containing such oils.

Although the domestication and development of each new oilseed crop requires a specific approach dependent on the characteristics of the plant species, the history of the crop and its potential uses, some generalisations can be made about the steps involved. Both for crop development (agricultural side) and product development (industrial side), four stages can be distinguished: exploration, examination, expansion and exploitation. A more detailed description of this concept, known as the '4-ex model', can be found in Chapter 1 of this thesis.

In 1986, the first of a series of projects on potential industrial crops in the Netherlands was launched. Around 40 oilseed species were evaluated for various agronomic characteristics as well as oil content and quality. After considering the industrial interest and agricultural potential, one of the species selected for further breeding research was *Dimorphotheca pluvialis* (L.) Moench. Seeds of this species contain ca 21% oil with approximately 60-65% dimorphelic acid ( $\Delta^9$ -hydroxy,10t,12t-octadecadienoid acid). The highly reactive hydroxydiene structure provides this fatty acid with a unique functionality and properties, making it potentially suitable for application in e.g. pharmaceuticals, surfactants, coatings, plastic foams, polymers, fragrances and flavours.

Although species of the genus *Dimorphotheca* have been known in the Netherlands as garden ornamentals for several centuries, the use of *D. pluvialis* for the production of seed oil is completely new. With regard to its use as an arable crop no breeding activities have been reported earlier, and as such the species should be considered as undomesticated. In the exploration of its potential as an arable oilseed crop, several constraints were identified, e.g. a long and unsynchronised period of flowering and seed ripening, poor seed retention and rather low oil content of the seeds. Hence, reported seed and oil yields were erratic. Seed retention seemed difficult to quantify, and preliminary observations revealed little variation for this character. Given the relatively short duration of the projects on which this thesis is based, attention was therefore focused on other important yield limiting factors: flowering synchronisation and oil content. Emphasis was laid on genetic improvement of these traits by means of selection. The efficiency of selection was examined by determination of the response to selection and estimations of the heritability.

For successful seed production, knowledge on the mode of reproduction of the crop is imperative. In the literature, *D. pluvialis* is described as a highly allogamous species, but little is known about the mode of pollen transfer. The influence of insects on several yield components was studied by comparing plant populations in the presence and absence of insects (Chapter 2). Exclusion of insects had a dramatic effect on the production of flowers and the duration of the flowering period. The total number of flowers at peak bloom was higher, and flowering continued longer in the absence of insects. Seed weight was somewhat higher, but seed set, seed yield and oil content were severely reduced under these circumstances, thousand seed weight was somewhat higher. The total seed and oil yield of insect-visited plots were, respectively, 4.5 and 5.5 times higher than those of insect-free plots. These results confirm the assumptions on the allogamous nature of the species and underline the importance of insect pollination for adequate seed yield in *D. pluvialis*.

Next to seed yield, oil content is a second important oil yield determining factor. High and stable oil yields of good quality are essential to provide industry with a constant supply. Three different populations of *D. pluvialis* were therefore subjected to mass selection for higher oil content (Chapter 3). After three cycles of selection at an intensity of 10%, for all three populations a significant increase in oil content was observed. Per selection cycle, an average gain in oil content ranging from 0.5% to 1.2% was achieved, depending on the population used. Realised heritabilities for this feature after three selection cycles in these populations ranged from 0.15 to 0.58. In Chapter 4, heritabilities for oil content were estimated from parent-offspring regression and half-sib family variance components. For this, forty plants were selected and progenies were tested twice, in two consecutive years. Heritability estimates from this experiment were moderately low: 0.34 from parent-offspring regression and 0.27 from variance components. If from this experiment 10% of the parental plants had been selected, an increase in oil content of 0.8% in one selection cycle would have been achieved. These values are in accordance with the values mentioned in Chapter 3. Although heritability estimates are specific for populations and environmental circumstances, it is likely that (mass) selection for increased oil content in general will be effective, particularly in the early generations. Considering the observed additive genetic variation in the populations, an average oil content of at least 30% seems feasible.

Particularly in combination with poor seed retention, the long, unsynchronised period of flowering and seed ripening is undesired. When a crop is harvested too early, yield losses occur due to incomplete setting and maturation of the seeds. When harvested too late, however, seed shattering will account for a severe reduction of seed yield. With regard to synchronisation of flowering, two main components can be distinguished; i.e. the synchronisation between plants and the synchronisation within plants. Synchronisation between plants is attained when plants of a population start flowering at the same time. Synchronisation within a plant is achieved when its flowers are produced in a short period of time. Both components are considered important for improvement of flowering synchronisation of the

crop. Therefore, the flowering of individual plants was studied by counting the open flowers at regular time intervals (Chapter 5). It appeared that the flowering process of individual plants can be described mathematically by a logistic curve, obtained by the regression of the cumulative number of open flowers plotted against time. The curve is characterised by three parameters, corresponding with the total number of flowers produced by the plant, the rate of flowering development and the day at which peak bloom was reached. From these parameters, subsequently two other characteristics could be derived, namely onset of flowering and duration of flowering within the plant. Similar to the method described for oil content, heritabilities of the flowering traits were estimated by using parent-offspring regression and half-sib family variance components analyses. Onset of flowering and peak bloom showed high ( $> 0.69$ ) heritabilities for both methods and both years, and total number of flowers showed moderate to high (0.30 - 0.90) heritability values. For these traits considerable progress may be expected from mass selection, particularly in the early selection generations. Duration of flowering showed low to moderate values (0.25 - 0.45), and thus for improvement of this trait methods other than mass selection should be considered.

Duration of flowering, total number of flowers and onset of flowering do not seem to be correlated (Chapter 5). Oil content and onset of flowering also seem to be uncorrelated (Chapter 4). Selection for either of these traits will most likely not influence the others.

Modern agriculture requires uniform plant populations. The currently available populations of *D. pluvialis*, however, most often originate from botanical gardens or gene banks and show considerable variation for morphological and agronomic traits. To improve morphological uniformity and to determine a preliminary ideotype for plant architecture, divergent mass selection for this character was carried out (Chapter 6). In order to minimise undesired side-effects due to assortative mating caused by variation in onset of flowering, selection for plant architecture was combined with selection for onset of flowering (earliness). Hence, six selection groups were distinguished: all possible combinations of two plant architecture types (erect and procumbent), with three earliness classes (early, middle and late). Three cycles of combined selection resulted in a significant response for both traits in both directions, even at a low selection pressure. In this experiment, selection for early flowering or procumbent architecture showed a better response and a higher heritability than selection for late flowering or erect plant architecture. The different plant architecture selections showed similar flowering development and seed yield. Therefore, from these results no conclusions on ideal architecture type with regard to breeding for increased yield could be drawn. However, for cultivation generally erect plant types are preferred. Earliness did have a significant effect on seed yield: early flowering types showed the highest yields. As the yield experiment was carried out only in one year, and genotype by environment interactions could not be assessed, no firm conclusions on ideotype with regard to earliness could be drawn. Nevertheless, (very) late flowering selections in general are undesired in the Netherlands because of an increased risk of experiencing unfavourable weather conditions during flowering and seed set.

*D. pluvialis* seems well adapted to the climatic conditions of north-west Europe, and fits well in a crop rotation system with annuals (Chapter 7). Its susceptibility to soil-borne diseases should be taken into consideration, but so far this has not caused major crop damage. Other pests and diseases seem to be easily controlled by agrochemicals. Improvement of resistance to several diseases can most likely be achieved by breeding, and deserves further attention. Genotypes with quick soil cover and improved harvest index may contribute to a higher potential seed production. Apart from further selection for increased oil content and flowering synchronisation, special attention should be directed towards improvement of seed retention. Even under optimal harvest conditions, seed losses of 20% were reported, entirely due to shattering. For good oil quality, relatively expensive methods for oil recovery have to be used. At present, oil of *D. pluvialis* seems particularly suitable for use in products with a high added value. However, many potential applications have not been explored yet. The unique structure and functionalities of dimorphecolic acid call for further research!

## Samenvatting

Wereldwijd, maar vooral in West Europa en de Verenigde Staten is de interesse voor akkerbouwgewassen met niet-voedsel toepassingen in de afgelopen decennia aanzienlijk toegenomen. Overschotten van de belangrijkste voedselgewassen en de industriële interesse voor hernieuwbare grondstoffen, hebben geleid tot R&D programma's gericht op de introductie van gewassen met industrieel toepasbare inhoudsstoffen. Met name plantaardige oliën die vetzuren met functionele groepen bevatten zijn aantrekkelijk als vervanger van minerale oliën die gebruikt worden in de fabricage van bijvoorbeeld smeermiddelen, oppervlakte-actieve stoffen, coatings en polymeren. Daarom is in de afgelopen jaren veel aandacht besteed aan het domesticeren van (veelal nogal wilde) soorten die dergelijke oliën produceren.

Hoewel de domesticatie en de ontwikkeling van ieder nieuw oliezaadgewas een specifieke aanpak vergt die afhankelijk is van de specifieke eigenschappen van de soort, de geschiedenis van het gewas en de mogelijke toepassingen, kunnen toch een aantal algemene aspecten genoemd worden met betrekking tot de te volgen procedure. Zowel voor wat betreft de ontwikkeling van het gewas (landbouwkundige kant) als het product (industriële kant), kunnen vier stadia worden onderscheiden: verkenning, onderzoek, schaalvergroting en exploitatie. Een uitgebreide beschrijving van dit z.g. '4-ex model' (naar de Engelse terminologie voor de vier stadia), is te vinden in Hoofdstuk 1 van dit proefschrift.

In 1986 werd in Nederland het eerste van een reeks projecten met betrekking tot potentiële industriële gewassen gestart. Ongeveer 40 soorten met oliehoudende zaden werden geëvalueerd op verschillende landbouwkundige eigenschappen, oliegehalte en oliesamenstelling. Na afweging van de industriële belangstelling en de landbouwkundige prestaties, werd uit deze gewassen o.a. *Dimorphotheca pluvialis* (L.) Moench geselecteerd voor verder veredelingsonderzoek. De zaden van deze soort bevatten ca. 21% olie met ongeveer 60-65% dimorphecolzuur ( $\Delta^9$ -hydroxy, 10t, 12t, octadecadiëenzuur). De zeer reactieve hydroxydiën structuur geeft dit vetzuur unieke eigenschappen en maakt het mogelijk geschikt voor toepassing in o.a. farmaceutische producten, oppervlakte-actieve stoffen, coatings, schuimplastics, polymeren, geur- en smaakstoffen.

Alhoewel soorten van het geslacht *Dimorphotheca* in Nederland al enkele eeuwen bekend zijn als siergewas, is het gebruik van *D. pluvialis* voor de productie van olie geheel nieuw. Met betrekking tot het gebruik als akkerbouwgewas is eerder geen veredelingsonderzoek beschreven, en als zodanig kan de soort als ongedomesticeerd worden beschouwd. In de verkenningfase werden verscheidene knelpunten geïdentificeerd; o.a. een lange, ongesynchroniseerde bloei- en zaadrijpingsperiode, een slechte vastzadigheid en het vrij lage oliegehalte van de zaden. Zaad- en olieopbrengsten waren derhalve zeer variabel. Vastzadigheid bleek moeilijk te kwantificeren, en in eerste instantie leek weinig variatie voor deze eigenschap te bestaan. Mede gezien de relatief korte duur van de projecten waarop dit



proefschrift is gebaseerd werd daarom de aandacht geconcentreerd op andere belangrijke opbrengst-beperkende factoren: bloeisynchronisatie en oliegehalte. De nadruk werd hierbij gelegd op genetische verbetering van deze eigenschappen door middel van selectie. De efficiëntie van selectie werd onderzocht door bepaling van de selectierespons en schattingen van de erfelijkheidsgraad.

Voor een goede zaadproductie is kennis van de wijze van reproductie van het gewas onontbeerlijk. In de literatuur wordt *D. pluvialis* omschreven als een in hoge mate kruisbestuivende soort, maar er is weinig bekend over de manier waarop pollenoverdracht plaatsvindt. De invloed van insecten op diverse opbrengstcomponenten van *D. pluvialis* werd bestudeerd door plantpopulaties onder aan- en afwezigheid van insecten met elkaar te vergelijken (Hoofdstuk 2). Het weghouden van insecten had een dramatisch effect op de productie van het aantal bloemen en de bloeiduur. Het aantal geproduceerde bloemen was hoger, en de bloei duurde langer in afwezigheid van insecten. Zaadgewicht was iets hoger, maar zaadzetting, zaadopbrengst en oliegehalte waren echter aanmerkelijk lager onder deze omstandigheden. De totale zaad- en olieopbrengst van door insecten bezochte veldjes waren respectievelijk 4.5 en 5.5 keer hoger dan die van insect-vrije veldjes. Deze resultaten ondersteunen de aannames omtrent het kruisbestuivende karakter van de soort en onderstrepen het belang van insectenbestuiving voor een goede zaadopbrengst bij *D. pluvialis*.

Naast zaadopbrengst is oliegehalte een tweede belangrijke olieopbrengst-bepalende factor. Hoge en stabiele olieopbrengsten van goede kwaliteit zijn essentieel om de industrie van een constante toevoer te voorzien. Drie verschillende populaties van *D. pluvialis* werden daarom onderworpen aan massaselectie voor verhoogd oliegehalte (Hoofdstuk 3). Na drie selectieronden met een selectie-intensiteit van 10%, werd voor alle drie populaties een significante stijging van het oliegehalte gevonden. Per selectieronde werd, afhankelijk van de gebruikte populatie, een toename in het oliegehalte van gemiddeld 0.5% - 1.2% gerealiseerd. Na drie selectieronden voor deze eigenschap varieerde de waarde van de 'gerealiseerde erfelijkheidsgraad' in deze populaties tussen 0.15 en 0.58. In Hoofdstuk 4 werd de erfelijkheidsgraad voor verhoogd oliegehalte geschat uit zowel ouder-nakomelingschapsregressie als uit 'half-sib'-familie variantiecomponenten. Hiervoor werden 40 planten geselecteerd, waarvan de nakomelingschappen twee maal, in twee opeenvolgende jaren, werden getoetst. De schattingen voor de erfelijkheidsgraad uit deze proef waren tamelijk laag: 0.34 uit ouder-nakomelingschapsregressie en 0.27 uit de variantiecomponenten. Indien in deze proef 10% van de ouderplanten zou zijn geselecteerd, zou een verhoging in het oliegehalte van 0.8% in één selectieronde zijn gerealiseerd. Deze uitkomsten komen redelijk overeen met de gevonden waarden in Hoofdstuk 3. Alhoewel schattingen van de erfelijkheidsgraad specifiek zijn voor populaties en milieu-omstandigheden, is het waarschijnlijk dat (massa-)selectie voor verhoogd oliegehalte in het algemeen effectief zal zijn, zeker in de eerste generaties. De gevonden beselecteerbare genetische variatie in de gebruikte populaties, wijst er op dat een gemiddeld oliegehalte van tenminste 30% haalbaar is.

Met name in combinatie met een slechte vastzadigheid is een lange, ongesynchroniseerde bloei- en zaadrijpingsperiode ongewenst. Bij vroege oogst ontstaan zaadverliezen door onvolledige zaadzetting en zaadrijping. Indien echter laat wordt geoogst, zal (voortijdige) zaadval voor een grote reductie van zaadopbrengst zorgen. Met betrekking tot bloeisynchronisatie kunnen twee componenten worden onderscheiden: de synchronisatie tussen en de synchronisatie binnen planten. Synchronisatie tussen planten wordt bereikt als alle planten van een populatie tegelijkertijd bloeien. Synchronisatie binnen planten wordt bereikt wanneer de bloemen van een plant in een kort tijdsbestek worden geproduceerd. Beide componenten worden als belangrijk beschouwd bij de verbetering van bloeisynchronisatie van het gewas. Daarom werd de bloei van planten bestudeerd door de open bloemen te tellen met regelmatige tussenpozen (Hoofdstuk 5). Het bleek dat de bloei van individuele planten wiskundig kan worden beschreven door een logistische curve, verkregen door regressie van het cumulatieve aantal bloemen per plant uitgezet tegen de factor tijd. Deze curve wordt gekarakteriseerd door drie parameters, corresponderend met respectievelijk het totaal aantal geproduceerde bloemen per plant, de snelheid van bloemontwikkeling en de dag waarop de piek van de bloei wordt bereikt. Uit deze parameters konden vervolgens nog twee andere worden afgeleid, namelijk begin van de bloei en bloeiduur van de plant. Op dezelfde manier als beschreven voor oliegehalte werden de erfelijkheidsgraden van deze bloeikenmerken berekend, zowel uit ouder-nakomelingschapsregressie als uit 'half-sib'-familie variantie-componenten. Begin van bloei en dag van piek bloei lieten bij beide methoden en voor beide toetsjaren hoge erfelijkheidsgraden zien ( $> 0.69$ ), en totaal aantal bloemen matige tot hoge ( $0.30 - 0.90$ ) waarden. Voor deze eigenschappen mag daarom aanzienlijke vooruitgang door massaselectie worden verwacht, vooral in de eerste generaties. Bloeiduur vertoonde lage tot matige heritability waarden ( $0.25 - 0.45$ ), en voor deze eigenschap zou derhalve een andere methode dan massaselectie moeten worden overwogen. Bloeiduur, totaal aantal bloemen en begin van bloei lijken ongecorrleerd (Hoofdstuk 5). Oliegehalte en begin van bloei lijken eveneens niet gecorreleerd (Hoofdstuk 4). Selectie op één van deze eigenschappen zal daarom de andere waarschijnlijk niet beïnvloeden.

De moderne landbouw vereist uniforme plantpopulaties. De momenteel beschikbare populaties van *D. pluvialis* zijn echter meestal afkomstig uit botanische tuinen of genenbanken en vertonen veel variatie voor morfologische en landbouwkundige eigenschappen. Teneinde de morfologische uniformiteit van populaties te verbeteren, en een voorlopig ideotype voor planthabitus te bepalen, werd divergente massaselectie voor dit kenmerk uitgevoerd (Hoofdstuk 6). Om ongewenste neveneffecten van 'assortative mating', veroorzaakt door variatie voor begin van bloei, te minimaliseren, werd selectie voor planthabitus gecombineerd met selectie voor begin van bloei (vroegheid). Aldus werden zes selectiegroepen onderscheiden: alle mogelijke combinaties van twee habitustypen ('opgericht' en 'plat') met drie vroegheidsklassen ('vroeg', 'middel' en 'laat'). Drie ronden gecombineerde massaselectie resulteerde in een significante respons voor beide eigenschappen in beide richtingen, zelfs bij een lage selectiedruk. In dit

experiment gaven selectie voor vroege bloei of platte habitus een betere respons en een hogere heritability te zien dan selectie voor late bloei of opgerichte habitus. De verschillende habitus-selecties vertoonden een zelfde bloei-ontwikkeling en zaadopbrengst. Op grond van deze resultaten kon derhalve geen conclusie worden getrokken omtrent de ideale planthabitus met betrekking tot zaadopbrengst. In het algemeen wordt voor de teelt echter een opgerichte planthabitus verkozen. Vroegheid had wel een significant effect op zaadopbrengst: vroege selecties hadden de hoogste opbrengsten. Omdat de opbrengstproef slechts in één jaar werd uitgevoerd, en genotype maal jaar interacties niet konden worden bepaald, kon geen definitieve uitspraak worden gedaan over het ideotype met betrekking tot vroegheid. Niettemin zijn in Nederland (zeer) laat bloeiende populaties ongewenst vanwege het verhoogde risico op slecht weer tijdens bloei en zaadzetting.

*D. pluvialis* lijkt aangepast aan het klimaat in noordwest Europa, en past goed in een rotatieplan met éénjarigen (Hoofdstuk 7). De vatbaarheid voor diverse bodempathogenen moet in acht worden genomen, al hebben deze tot dusverre niet geleid tot grote schade aan het gewas. Andere ziekten en plagen lijken voorlopig afdoende te bestrijden met agrochemicaliën. Verbetering van resistentie tegen diverse pathogenen middels veredeling lijkt haalbaar en verdient aandacht. Genotypen met een snelle bodembedekking en een hogere oogstindex kunnen mogelijk de potentiële zaadproductie verder verhogen. Naast verdere selectie voor verhoogd oliegehalte en verbeterde bloeisynchronisatie dient speciale aandacht uit te gaan naar verbetering van de vastzadigheid. Zelfs onder 'optimale' oogstomstandigheden werd een zaadverlies van 20% geconstateerd, geheel veroorzaakt door gebrek aan vastzadigheid. Voor olie met een goede kwaliteit moeten relatief dure methoden voor oliewinning worden gebruikt. Op dit moment lijkt olie van *D. pluvialis* voornamelijk geschikt voor gebruik in producten met een hoge toegevoegde waarde. Veel mogelijke toepassingen zijn echter nog niet onderzocht. De unieke structuur en eigenschappen van het vetzuur vragen om verder onderzoek!

## Nawoord

Na jaren van noeste arbeid is het er dan toch van gekomen: het proefschrift is af. Dat ging niet vanzelf, en veel mensen hebben in meer of mindere mate een bijdrage geleverd aan de totstandkoming ervan.

Het begon in 1991, toen ik als wetenschappelijk onderzoeker ging werken bij de sectie 'Potentiële Gewassen' van het CPRO-DLO. In die tijd was Loek van Soest sectieleider, en stuwende kracht achter het industriële gewassen onderzoek in Nederland. Hij was de initiatiefnemer en coördinator van de door de EG medegefinancierde projecten VOICI en VOSFA, waarbinnen het in dit proefschrift beschreven onderzoek is uitgevoerd. Met name in die eerste jaren is menig pittige discussie gevoerd over het 'hoe en waarom' van het onderzoek aan de diverse industriële gewassen. Het was een turbulente maar inspirerende tijd, gekenmerkt door een bijzonder soort saamhorigheid binnen de sectie. Loek en andere collega's uit die tijd, bedankt!

Voor het verzamelen van de gegevens voor dit proefschrift zijn talloze kas- en veldproeven uitgevoerd. Alle mensen van de proefvelddienst op 'De Haaff' in Wageningen en op de 'Prof. Broekmahoeve' in Lelystad die een bijdrage hebben geleverd aan het zaaien, onderhouden en oogsten van de proeven en niet te vergeten het schonen van het zaad, wil ik hierbij bedanken voor hun inzet.

Met name assistent Ite Nieboer, maar ook de toenmalige studenten Evelien Vlaardingerbroek, Ivonne Elberse en Edwin Nuyten wil ik bedanken voor de vele uren die we samen in de kas en op het veld hebben doorgebracht om de benodigde gegevens te verzamelen. Weer of geen weer, we hebben tientallen proeven gezaaid, honderden planten geselecteerd, duizenden planten beoordeeld, tienduizenden bloemhoofdjes geteld en miljoenen zaadjes geoogst. En dat alles is nu samengevat in dit ene boekje...

Teneinde te kunnen selecteren op oliegehalte was de ontwikkeling van een 'snelle' screeningsmethode van de zaden noodzakelijk. Rob Frankhuizen en collega's van RIKILT-DLO hebben daar een grote bijdrage aan geleverd, waarvoor bedankt. Naast Ite Nieboer heeft ook Marie Lubberts vervolgens vele dagen achter het bewuste NIRS-apparaat doorgebracht om de grote hoeveelheid zaadmonsters te screenen, waarvoor ik haar zeer dankbaar ben.

Yde Jongema en Willem Jan Boot van de vakgroep Entomologie van de LUW ben ik zeer erkentelijk voor het determineren van de vliegende insecten die we op het gewas tegenkwamen.

Met de partners binnen de projecten zijn veel openhartige gesprekken gevoerd over de onderzoeksresultaten. In deze informele sfeer was het mogelijk om nuttig gebruik te maken van de uitgewisselde informatie, wat de resultaten zeker ten goede is gekomen. Iedereen die daarbij betrokken was wil ik daarvoor hartelijk bedanken.

Bij de statistische verwerking van de verzamelde proefgegevens is Paul Keizer van onschatbare waarde gebleken. Zijn grote inzet, zijn eindeloze geduld en zijn immer welwillend luisterend oor waren zeer inspirerend. Bedankt Paul!

Bij het schrijven van de publicaties en uiteindelijk dit proefschrift, heb ik zeer veel steun gehad van mijn 'college van wijze heren': Wouter Lange, Oene Dolstra en Piet Stam. Wouter was eerst co-promotor, maar besloot die taak na enige tijd over te dragen aan Oene. Gelukkig was hij wel bereid om, ondanks zijn vervroegde pensionering, officieus als co-promotor aan te blijven. Met name zijn aanbevelingen op het gebied van taalgebruik in wetenschappelijke publicaties waren bijzonder waardevol. Oene heeft reeds vanaf het begin van het project uitstekende wetenschappelijke ondersteuning verleend. Eerst, inherent aan zijn persoonlijkheid, bescheiden op de achtergrond. Later, als co-promotor geheel verdiend wat meer op de voorgrond. Piet was een waardig promotor door met name de grote lijnen van het geheel goed in de gaten te houden. Zijn commentaar was veelal bescheiden in kwantiteit, doch immer hoog van kwaliteit. Door de combinatie van hun persoonlijke kwaliteiten vormden ze met z'n drieën een uitstekend begeleidingsteam, waarmee ik plezierig heb vergaderd en waarvan ik zeer veel heb geleerd. Heren, mijn dank is groot.

De fraaie pentekening van *Dimorphotheca pluvialis*, die in Hoofdstuk 1 is opgenomen, is gemaakt door Dorothee Becu.

Tenslotte wil ik (ex-)collega's, vrienden, familie, en in het bijzonder Hans bedanken voor hun morele steun. Die is voor mij van onschatbare waarde geweest.

Lysbeth

## **Curriculum vitae**

Lysbeth Hof werd op 4 februari 1964 geboren te Drachten, als jongste in een gezin met drie meisjes. In 1982 behaalde zij het Atheneum-B diploma aan 'Het Drachtster Lyceum', en in datzelfde jaar begon ze met de studie Plantenveredeling aan de Landbouwhogeschool te Wageningen (later Landbouwuniversiteit en inmiddels Wageningen Universiteit). Tijdens deze studie is zij twee maal een half jaar naar het Scottish Crop Research Institute te Dundee (UK) gegaan, voor respectievelijk een stage met als hoofdonderwerp de voortijdige vruchtval van zwarte bessen, en een afstudeervak betreffende de ontwikkeling van inoculatietechnieken voor de schimmel *Elsinoe veneta* op framboos. Tussendoor heeft zij op de Vakgroep Tuinbouwplantenteelt van de LUW onderzoek gedaan naar de optimalisatie van weefselkweekmethoden voor seringen. Na afronding van haar studie is ze achtereenvolgens werkzaam geweest als onderzoeker op een weefselkweeklaboratorium voor met name orchideeën te Assendelft, en als assistent onderzoeker op de Vakgroep Plantenfysiologie van de LUW, waar ze o.a. cytokininegehalten van bladponsjes van *Kalanchoe* heeft bepaald. Van 1991 tot 1998 heeft ze op het Centrum voor Plantenveredelings- en Reproductieonderzoek (CPRO-DLO, inmiddels opgegaan in Plant Research International) te Wageningen, veredelingsonderzoek gedaan aan nieuwe gewassen met voor de industrie mogelijk interessante inhoudsstoffen. Eén van deze gewassen was *Dimorphotheca pluvialis*, waarvan de belangrijkste onderzoeksresultaten als basis hebben gediend voor dit proefschrift. In deze periode is ze tevens getrouwd met Hans van der Wal, en werden hun dochter Gemma (1995) en zoon Gijs (1997) geboren.