Cultivation and breeding of *Digitalis lanata* in the Netherlands

C MASTENBROEK

From the Stichting "Kruidentuin te Doornspijk", Elburg, The Netherlands

SUMMARY After the second world war Marshall Aid funds were used to establish a cooperative organisation for growing, drying, and selling *Digitalis lanata* (and other medicinal, aromatic, and culinary herbs) in the Netherlands. The crop is sown in mid April and the fully mechanised harvest of the leaves takes place from September to late November. The leaves are dried for 10-12 hours at 50°C maximum. The aim of breeding trials is to improve leaf production, erect leaf attitude, resistance to *Septoria* leaf spot and to bolting, and a higher dry matter and digoxin content.

Cultivation

It is cheaper for the pharmaceutical industry to extract digoxin from the dried leaves of *Digitalis lanata* than to synthesise the drug chemically. *D lanata* (Fig. 1a) is called the woolly foxglove because the calyx of the flower is thickly covered with white glistening slightly sticky hairs (Fig. 1b). *Digitalis purpurea*, which is native to north west Europe, was grown for the extraction of digitoxin until the medical superiority of digoxin was demonstrated. Digoxin only occurs in *D lanata*, which is not native to north west Europe; its natural habitat is south east Europe.

After the second world war the Dutch government used some Marshall Aid funds to promote the cultivation of *D lanata* (and other medicinal plants and aromatic and culinary herbs). Several cooperative drying plants were established; three of these are still active. They are situated in the north east, centre, and south east of the Netherlands. In 1984 230 hectares of *D lanata* were harvested and dried. Contracts with growers are arranged by the three regional drying factories and a central cooperative VNK (Verenigde Nederlandse Krudencoöperatie, United Dutch Herb Cooperative) organises contracts with and delivery to the pharmaceutical industry.

D lanata is cultivated on peaty, sandy, and clay soils. The seeds are drilled in mid-April with machines adapted to sow very small quantities (2 kg per hectare) of tiny seeds. Germination is very slow, and it takes about two months before the seedlings

develop sizeable leaves. The leaves (Fig. 2) are harvested from the end of September till the middle of November or even later, depending on weather conditions. Harvesting is completely mechanised. The mean yield of fresh leaves is about 30 tonnes per hectare. The freshly cut leaves are immediately transported to the drying factories. After weighing they are coarsely cut, washed to remove soil particles, and dried for 10-12 hours at 50°C until their moisture content is 8-10%. The dried product, around 5 tonnes per hectare, is pressed into polyethylene bags (35 kg per bag) and exported mainly to the United Kingdom, the United States of America, Germany, and France.

Breeding

The growers, drying plants, and pharmaceutical industry all have an interest in the improvement of the inherited properties of the species. Their various priorities are set out below.

THE GROWERS

The yield of fresh leaves is the basis for payment. The farmer wants to grow a variety that produces a heavy leaf canopy. The first step in breeding, therefore, is selection of individual plants with many broad and long leaves. Until about 10 years ago the material cultivated had been obtained through natural accession from a botanical garden and did not have outstandingly large leaves (Fig. 3a). Individual plants with larger than average leaves were selected and grown for their seed. In the next generation the plant type appeared to have been somewhat improved. Variation was still apparent and this was

Requests for reprints to Dr C Mastenbroek, De Terminator 8, 8251 AD Dronten, The Netherlands.

Fig. 1 (a) *Digitalis lanata* in full bloom (photograph provided by Professor F L Meijler) and (b) woolly hairs on flower buds.

again exploited for the selection of better plants. Selection over three consecutive generations has considerably improved the plant type (Figs. 3b and c). It should be borne in mind, however, that in a well established production field more than 1 000 000 plants are competing for room, light, and nourishment, and this competition means that plants of the same genetic constitution will have a different appearance when they are grown in closed stands and as spaced plants in nurseries. The yields of individual plants will also differ considerably according to their growing conditions; nevertheless there is evidence that selection has improved leaf yield by about 15%.

The leaf attitude of the original accession tended to be prostrate, and some of the lower leaves remained attached to the plant after harvesting (Fig. 4). The leaves left behind can only be used as green manure and represent a loss in income to the grower. An upright leaf attitude is therefore an advantage. Selection has produced a plant type with a strikingly erect leaf habit. This improvement is more apparent when *D. lanata* is grown in nurseries of spaced plants rather than in close stands in production fields. Plants with erect leaves will tend to produce a cleaner harvested

product since the more upright the leaves are the less the driver of the cutting machine will be tempted to make a deep cut which harvests soil particles too.

Resistance to diseases makes measures to prevent or to check damaging attacks less necessary or even superfluous. Thus when resistant varieties are grown production costs for the same yield are lower. In commercial production only one fungus, *Septoria digitalis* (Fig. 5a), produces damage sufficient to require chemical control. In comparative trials the fresh leaf yield of the very susceptible original accession was reduced by as much as 20% between mid-September and the end of October when chemical control was not used (Fig. 5b). Spraying twice during the growing season costs about £125 per hectare. Selection of plants which have a high degree of natural resistance to disease has produced a variety which does not need to be sprayed with fungicides.

Resistance to bolting is important because it saves labour. Plants that develop stems with flowers and fruits in the first year are called bolters. Because the leaves contain much more digoxin than other parts of the plant, the raw material for extraction should consist of leaves only. Growers should undertake to remove bolters from their crops. This can only be done

Fig. 2 Mechanised harvest of leaf crop in commercial cultivation.

by hand. Thus they prefer to cultivate a variety which does not have a tendency to bolt. Bolting is stimulated by exposure of the young plants to low temperatures. This encourages the plants to develop flowers. The amount of stimulation required to initiate bolting depends on the plant's natural resistance to bolting. Selection for this feature is not difficult. When seeds are sown in cold frames in the first days of April and the seedlings are transplanted at the beginning of June the percentage of plants that bolt (Fig. 6) is much higher than that found in field crops and in comparative yield trials which are normally drilled around the middle of April. The exclusion from further selection of all bolting plants, including those in which bolting is only incipient, has produced a high level of resistance to bolting in only a few years.

THE DRYING FACTORIES

An upright leaf attitude produces a cleaner crop.

Dry matter content—The cost of drying the leaves by means of natural gas is high. The mean dry matter content of the leaves is about 20%, and the moisture

content has to be brought down from 80% (or more if the product has been washed) to 8–10%. An increased dry matter content reduces the cost of drying. There is genetic variation in dry matter content between individual plants and their seed progenies, but non-genetic variation between individual plants caused by external conditions may also be large. This makes effective selection for this character rather difficult. Nevertheless, plants with a higher dry matter content have been selected. It is difficult, however, to combine a high dry matter content with a high yield of fresh leaves and a compromise is necessary.

THE PHARMACEUTICAL INDUSTRY

Digoxin content influences production costs. As long as the price of the raw materials is not related to its digoxin content, the pharmaceutical industry will benefit from an increased digoxin content. A decrease in the content of compounds that resemble digoxin (63 glycosides have been found in *D lanata*) would also simplify purification. To select for a higher digoxin content individual plants are assessed

Fig. 3 (a) Plants of original accession and (b) improved selection. (c) An outstanding individual plant.

by paper chromatography. This is not a reliable method, but it is quick, simple, and cheap; and this is important since several hundred plants have to be tested in two to three weeks. In recent years samples of selections in yield trials have been investigated by high performance thin layer chromatography (Fig. 7). The digoxin content of dried leaves of the variety now in cultivation is around 2.5–3.0 parts per thousand, which is about 50% higher than the content of the original accession.

BREEDING PROCEDURE

Year 1—Selection of individual plants in spaced stands for characteristics described above in a nursery under heavy *Septoria* pressure. Only about 20 out of 10 000 plants are retained for further testing.

Year 2—Seeds are harvested from individual se-

lected plants.

Year 3—Seed progenies are tested for relevant characteristics in comparative yield trials (Fig. 8) in two replicate plots on clay and sandy soil. About 10 of the better progenies are retained.

Year 4—Repeated testing of 10 progenies as in year three. If one or two perform better than the standard over the two years, they are retained.

Year 5—Retained selections are sown for seed production.

Year 6—Seed production.

Year 7—Testing of seed progenies as in years 2 and 3 but on more plots and at different harvest dates. If only one selection performs satisfactorily, it will be used in commercial production from **Year 8** onwards, after which further seed multiplication will take place.

A new selection cycle is initiated each year, starting from progenies that are outstanding overall or for particular characteristics.

Fig. 4 Recumbent leaves remain after harvesting.

Seed production

D. lanata is self-fertile. The pollen is sticky, and selfing can be achieved by moving a small soft brush around inside the flowers. Flowers which have been treated in this way must be covered (bagged) to exclude bees and thus prevent cross pollination.

In the breeding work that I describe, however, the plants are not bagged. Bees are allowed to cross pollinate selected plants retained from year 1 for seed production in year 2. Plants with particular characteristics are grown in isolated plots to prevent cross pollination between groups. Seed production in year 6 and in successive years also takes place in isolated plots and fields.

Large numbers of bees are attracted by the glistening droplets of nectar at the bottom of the flower (Fig. 9). When the crop is in bloom bees carry the sticky pollen from plant to plant. This cross pollination produces heterogeneous progenies, and progeny produced by cross pollination of an outstanding plant is as a rule less outstanding than the mother-

Fig. 5 (a) Leaf spot disease caused by *Septoria digitalis* and (b) heavy damage in a susceptible variety.

plant itself. The genetic contamination of outstanding plants can be prevented by raising several consecutive generations by selfing, but inbreeding depression may result. This has not so far been investigated.

Natural pollination by bees certainly brings about a high degree of inadvertent crossing between outstanding plants in year 2. Some of the heterogeneous plants produced may have new and favourable combinations of genes. Such outstanding individuals can be selected in year 1 of each new selection cycle. The very best plants may be outstanding simply because they are cross bred. If this is true, it follows that genetic segregation in the sexual progenies is unavoidable and that some genetic deterioration will take place. This may indicate that selection should continue to maintain the favourable inherited characteristics of the variety. Investigations into this question have been started, but it will take several years before a conclusion can be drawn.

Seed production may be damaged by the grey mould *Botrytis cinerea*, a widely distributed fungus,

Fig. 6 Bolting plants flower in the first year.

Fig. 7 High performance thin layer chromatography densitograms showing a high peak in 23 digoxin in a selected variety and in the standard 1 (TNO, Zeist, The Netherlands).

Fig. 8 Comparative yield trial.

Fig. 9 Bees bring about cross pollination.

Fig. 10 A healthy stem with fruits producing many seeds (left) and a Botrytis-diseased stem producing no seed (right).

which can attack the inflorescence of *D. lanata*. In severe cases almost all the seed is lost. The fungus is only weakly pathogenic, but starting from dead flowers that remain stuck in the hairs on top of the young fruits or in the leaf axils, it may invade the stems. Growth of the fungus within the stem (Fig. 10) causes all the seed in an attacked inflorescence to be lost. The attack is favoured by rain, high air hu-

midity, and several days of high temperatures. *D. lanata* has no natural resistance to this disease, but Ronilan (vinchlozoline) which was developed to control the fungus in french beans is very effective in *D. lanata* if it is sprayed once a week (or twice a week when conditions are moist and warm) during the 6–8 week flowering period and once or twice after flowering.