Genetic, cytological and physiological aspects of seed yield in perennial ryegrass (*Lolium perenne* L.)

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Stellingen behorend bij het proefschrift 'Genetic, cytological and physiological aspects of seed yield in perennial ryegrass (*Lolium perenne* L.)' van Anjo Elgersma, te verdedigen op vrijdag 2 november 1990 te Wageningen.

This work was performed at the Foundation for Agricultural Plant Breeding SVP in Wageningen, the Netherlands, from June 1984 till December 1988.
The work was supported by the Commodity Board for Arable Products in the Hague, the Netherlands.

ABSTRACT

Seed yield in perennial ryegrass is low and unpredictable. Breeding for a high stable seed yield is difficult, as seed yield is a complex trait with a low heritability. The aims of this study were to identify selection criteria for high seed yield and to gain more insight in the biology of seed reproduction in perennial ryegrass. Genotypic and temperature effects on pollen tube growth rate were detected. Cytological studies of seed development, ovule degeneration and seed abortion demonstrated that non-productive florets occurred at all positions within the ear. Abscission layer development and the mechanism of seed shattering were similar in genotypes with different seed retention.

Indirect selection for seed yield would be more efficient, if correlated traits with a high heritability could be identified, either in spaced plants or in crops grown in drilled plots. Significant and consistent differences for seed yield per plot were found among nine perennial ryegrass cultivars over three years at two locations. These differences were not correlated with thousand-grain weight, other seed yield components or crop physiological traits assessed in drilled plots. Spaced plants within each cultivar differed greatly for many traits. Broad-sense heritabilities were high for all spaced-plant traits, but narrow-sense heritabilities were only significant for some traits. No spaced-plant traits were identified that correlate with seed yield in drilled plots.

Keywords: Lolium perenne, plant breeding, perennial ryegrass, seed yield components, pollination, fertilization, seed abortion, seed shattering, cultivar-environment interaction, dry matter partitioning, heritability, spaced plants, drilled plots

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CONTENTS

GENERAL INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is the most common fodder grass in Northwestern Europe. It is also widely used in other areas with a temperate climate and sufficient rainfall, e.g., New Zealand and parts of the USA. Its qualities are high persistence and dry matter production, good digestibility and palatability and rapid establishment after sowing. Negative features are susceptibility to drought and lack of winterhardiness. Perennial ryegrass is mostly used as a fodder crop, but cultivars for turf have also been developed recently. It is also used for green manure, especially tetraploid varieties.

Perennial ryegrass is propagated by seed. At the beginning of this century, imported seed often proved less suitable for Dutch conditions. Native seed was collected until 1930, but the quality was not always good (van Dijk, 1966). Until 1957, seed of good old pastures was recommended, but was difficult to obtain. The production of grass seed as an arable crop was introduced into Dutch agriculture in the fifties. Since then the acreage has increased to over 25,000 ha in 1990, consisting of about 11,000 ha perennial ryegrass. About half of the seed harvested in the Netherlands is exported (100 million guilders annually). Apart from the economical value of the marketed seed, grass seed crops are very valuable in arable farming. Nowadays, crop rotation is very narrow which leads to many soil-bound problems (nematodes, poor soil structure, low organic matter content, etc.). Grass seed crops have a very fine developed and intensive rootsystem which improves the structure and increases the organic matter content of the soil (Elgersma, 1988).

Grass breeding started in Wales before the second World War and major attention was focused on the relation between maturity, growth habit and persistence. Early cultivars had an erect growth habit and low persistence. They had a higher seed-yielding ability than late-heading cultivars. After the second World War, grass breeding developed rapidly. In spite of the low seed yields of late varieties, breeding for seed yield did not receive very much attention, because the breeders were afraid to sacrifice the high herbage quality of the late varieties (van Wijk, 1980). Seed production also increased anyway due to better agronomic practices. However, during the sixties, breeding for seed yield started to receive more attention (Griffiths, 1965; Lewis, 1966; Bean, 1972). Breeders realized, that continuous breeding for lateness and leafiness could impair the economics of seed production. Nowadays, a large number of technically good grass cultivars with slight differences in vegetative qualities are available, and the economics of grass-seed production ultimately decides the commercial succes of a cultivar (van Wijk, 1980).

The growing interest in genetic variation for grass seed production is illustrated by the following events: in 1978 the International Herbage Seed Production Research Group (IHSPRG) was founded. In 1981 a meeting of the Fodder Crops Section of Eucarpia, the European Breeders Association, was devoted to seed production in herbage cultivars. In 1983 the Journal of Applied Seed Production was released. In 1985 a symposium on Floret Site Utilization in Grasses was organized in the

Netherlands and in 1987 a conference on Seed Production in grasses and forage legumes was held in Denmark. The Commodity Board of Arable Products in the Netherlands funded a research program entitled "Genetic variation for seed yield in grasses", that was carried out at the Foundation for Agricultural Plant Breeding (SVP) in Wageningen from 1984 to 1988. The results are presented in the present thesis.

The aims of this study were to identify selection criteria for high seed yield and to gain more insight in the biology of seed reproduction in perennial ryegrass. Seed yield is a complex trait with a low heritability. Selection for seed yield can be made more efficient if traits in spaced plants or in a crop grown in drilled plots are identified that are suitable for indirect selection for seed yield per plot. Such traits should be easy to assess and should have a high heritability and a high correlation with seed yield per plot. Selection for such traits should not impair vegetative qualities. Therefore, in nine cultivars seed yield and its components were studied both on a spaced-plant level (the initial situation in breeding) and on a drilled plot level (the final situation in seed multiplication).

The importance of pollination, fertilization, seed development, abortion and seed shattering for floret site utilization and seed yield were discussed in a literature review (Chapter 1). Detailed studies were then made of these processes. Pollination studies were conducted in greenhouses with controlled temperatures (Chapter 2). Cytological studies of fertilization, seed development, ovule degeneration and embryo abortion and of seed shattering were carried out on spaced plants grown in the field (Chapters 3 and 4). In a large field experiment nine diploid cultivars were studied. Drilled plots were harvested for seed over a three year period at two locations (Chapter 5). Growth analyses were performed in drilled plots to gain more insight in the physiology of the seed crop and to study the components that determine seed yield (Chapter 6). Spaced plants were observed simultaneously. They served as a source of open-pollinated progeny, which was used for parent-offspring regression and heritability estimations (Chapter 7). They were also used to obtain information about variation within cultivars and to define the phenotypic spaced-plant traits upon which selection for high seed yield should be based (Chapter 8).

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CHAPTER 1

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Floret Site Utilization in Grasses: Definitions, Breeding Perspectives and Methodology[1]

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ABSTRACT

The seed yield of grasses needs improvement, and seed yield should become a more important selection criterion in grass breeding. Seed yield depends largely on the degree of floret site utilization (FSU). This term is extensively discussed in this paper and several definitions are reviewed. A distinction is made between biological and economical FSU. Causes of variation in FSU and various determination methods are discussed. Suggestion for breeding for increased FSU are also given.

Additional index words: **Seed yield, pollination, fertilization, seed set, seed development, seed harvest, abortion, shattering, harvest losses.**

INTRODUCTION

The success of a herbage cultivar in commercial production not only depends on its forage attributes, but also on its ability to produce seed. In forage varieties, characteristics such as high vegetative production, persistency and quality are of importance to the farmer. The seed producer, however, desires high seed yields of good quality.

There are two alternatives for maximizing seed yields:

1. Cultural manipulation of the grass seed crop to maximize development of flowering stems and fertile florets.
2. Breeding for improved seed production jointly with forage production when developing new varieties.

Breeding perspectives for higher seed production are the focus of this paper.

Floret Site Utilization (FSU), Definitions and Components

Grass seed crops have a high yield potential, but this potential is never fully realized. Griffiths et al. (1973), Hebblethwaite (1977) and Burbidge et al. (1978) found realized yields to be much lower. Based on seed yield components, the theoretical production or potential yield can be calculated as inflorescences m^{-2} x spikelets/inflorescence x florets/spikelet x FSU x average seed weight. Owing to the negative feedback on forage production, it is not desirable to increase the size of the reproductive system, i.e. florets m^{-2}. Instead, the efficiency should be increased (Bean, 1972), which can be achieved by improving the FSU. The meaning of this term, however, is not clearly defined. Bean (1972) defines efficiency of the reproductive system as the percentage of flowers which produce seed, and the size to which these seeds develop. Several other terms have been used to describe FSU. For example, seed set, floret fertility or fertility index have been used. However, some of these last terms are already used for other traits, being components of the total "floret site utilization". For a full understanding of FSU it is necessary to analyze which processes occur from the time of anthesis until determination of the final seed yield. It is important to know how these processes are influenced by environmental and genetic factors, and by genotype x environment interactions.

In a *biological* sense, FSU can be defined as the percentage of florets, present at anthesis, resulting in a viable seed. For seed growers, the percentage and quality of the seeds that can be *harvested* is of interest. After harvesting and cleaning, the commercial seed yield can be expressed as a percentage of the potential seed yield. In an *economical* sense, FSU can be defined as the percentage of florets present at anthesis which contribute to the harvested seed. Economical FSU results from the following processes: pollination, fertilization, seed set, seed development, harvesting and cleaning. During each of these processes losses may occur (Table 1).

Pollination

The term 'pollination' is used to describe processes occurring from the time of anther dehiscence until the pollen reaches the stigma. Pollen grains on the stigma have a mutually stimulating effect on pollen germination. Reduction of the number of pollen grains on the stigma may reduce fertilization. Early lodging limits pollen transport and successful pollination. On the other hand, uniform flowering and high pollen production favor pollination. Pollination is influenced by environmental factors.

Fertilization

In the progamic phase of fertilization, seed set may be reduced due to non-viability of pollen grains or incompatibility. Non-viable egg cells may also reduce FSU.

Pollen grains of grasses have a low retention of viability, especially at low humidity. Jones and Brown (1951) stated

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Table 1. Processes occurring after anthesis and associated losses that reduce floret site utilization.

Florets at anthesis	Losses
Pollination	Empty florets
Fertilization	Empty florets
Seed set	Empty florets Early abortion
Seed development	
- Early growth stage	Abortion
- Food reserve accumulation stage	Diseases Abortion
- Ripening stage	Diseases Shattering
Harvesting	Shattering Damage
Cleaning	Empty florets Light seeds Heavy seeds
Seed yield	

that the poor seed set observed in some grass species may be due to the susceptibility of stigmas to damage under high temperatures and to desiccation of the pollen. Stigma withering in grasses begins a few hours after pollination. On the stigma, about 60-80% of the grass pollen germinates. The germination percentage increased with greater maturity of pollen and stigma (Watanabe, 1961). Hebblethwaite and Hampton (1981) mentioned that possibly not all florets are potentially fertile, either because of pest damage (Johnston, 1960) or because they are morphologically sterile and incapable of developing seed (Johnston, 1960; Hill, 1980). Johnston (1960) found up to 10% morphological sterility in flowers of *Dactylis glomerata*. Part of the 'sterility' in *Bromus inermis* and *Agropyron* spp. is genetically determined; the environment also strongly influences the percentage of flowers that set seed (Knowles and Baenziger, 1962).

Fertilization may be reduced due to self-incompatibility, which is quite common in grasses. In ryegrass, for example, there is a 2 loci gametophytic system, but under certain conditions some self-fertilization can occur. Probably the absence of other pollen favors self-fertilization. Poor seed set might occur when synthetic varieties are based on a few clones.

Seed Set

According to Hill (1980), the term 'seed set' describes the early growth of the embryo and endosperm. "Set" is indicated by the presence of cell division following successful fertilization.

As shown by Hill (1971) and Burbidge et al. (1978), approximately 60% of all florets are capable of being fertilized. The position of a floret in the inflorescence influences the probability of seed set (Anslow, 1964). In ryegrass, abortion may occur after fertilization up to approximately 21 days after anthesis. Soon after fertilization, cell division can be disrupted resulting in a misshapen ovary. At a later stage, seed development is often resumed. In other cases, cells disintegrate and the entire ovule collapses (Hill, 1980).

It is not clear whether abortion occurs more often after self-fertilization. Burbidge et al. (1978) noticed in *Lolium perenne* that many more seeds were set than were harvested, even when the crop was not lodged. They mentioned two possible reasons for abortion of developing seeds: hormonal inhibition of seed growth and competition for assimilates.

Seed Development

Hyde et al., (1959) distinguished three stages in seed development in ryegrasses:

Stage 1: the growth stage, duration 10 days after pollination. **Characteristics:** rapid increase in seed weight, high seed moisture content and non-viability.

Stage 2: the food reserve accumulation stage, duration a further 10-14 days. **Characteristics:** a threefold increase in seed dry weight. Seeds attain full viability.

Stage 3: the ripening stage, duration 3-7 days. **Characteristics:** dry weight remains approximately constant, but moisture content falls from about 10% to equilibrium with the atmosphere.

The stage of seed development affects three important aspects of seed quality: viability, seedling vigor and storage life (Hyde, 1950). Perennial ryegrass seed harvested 14 days after pollination would be viable, but would give rise to seedlings with poor vigor as high seedling vigor is not present until about 24 days after pollination. Immature seeds deteriorate rapidly in storage.

A study by Hill (1971) with perennial ryegrass has shown that different genotypes may have different patterns of embryo maturation. Furthermore, genotypes differ with respect to dehydration and the time taken to attain harvest ripeness (Hill, 1980). Cultural practices have a major effect on seed development.

During seed development, shedding losses occur. Besides trying to increase seed set, loss of viable seed can also be reduced. Burbidge et al. (1978) did not consider shedding to be a major factor, which contrasts with the results of Stoddart (1964). According to Stoddart (1964), one of the principal sources of yield loss in grass seed crops is the amount of seed shed from the inflorescence. Environmental factors, such as heavy winds, affect shedding. Spread in ripening time also promotes shattering. In *Lolium multiflorum*, genetic variation for seed retention was found between populations (Harun and Bean, 1979). Several authors reported improved seed retention resulting from breeding for this character (McWilliam, 1980; *Phalaris aquatica* (syn. *tuberosa*); Bean, 1969; *Phleum pratense*; Falcinelli et al., 1984; *Dactylis glomerata)*.

Seed retention does not affect forage qualities and seems to be a very desirable character in grass seed crops. When growth regulators are introduced into commercial seed production, seed retention will become even more significant.

Harvesting

Not all seed that is produced can be harvested because of spread in ripeness, lodging and harvest losses. Within a seed crop, differences in ripeness exist within and between spikelets, between inflorescences and between plants. The apical florets in the spikelet, the apical part of an inflorescence and the oldest inflorescences ripen fast. The harvest time is a compromise between seed yield and quality. When the crop is harvested too early, many immature seeds are harvested, resulting in:

-drying costs
-increased risk of damage
-cleaning losses (light seed)
-loss of quality (light seed with low seedling vigor)

If harvest is late, many seeds are lost through shedding. At first, apical seeds will shatter which contain light seed. Then, the oldest inflorescences which carry the heaviest and the greatest number of seeds also start to shed. Bonin and Goplen (1963) found that within clones of *Phalaris arundinacea* shattered seeds were heavier than non-shattered seeds. Jensen (1976) found that shed seeds had a lower moisture content than non-shattered seeds. As the time of harvesting differs more from the mean ripeness date, the risk of genetic shift also increases (Davies, 1954).

A lodged crop is difficult to harvest. Morover, new vegetative tillers may grow through the lodged inflorescences and hamper harvesting. Seed quality decreases when the seeds are attacked by fungi. In a lodged crop, shattering is reduced. The harvest method, i.e. combining or swathing, affects seed yield losses. Seeds can be damaged, resulting in decreased quality. Harvest losses are greatly influenced by weather conditions.

Cleaning

After harvesting and drying, the seed is cleaned. Normal or "heavy" seed is separated from dust, sand, stalks, entire spikelets, empty florets, light seed and weeds. The cleaned seed is weighed, providing the final (economical) seed yield. The number of clean seeds can be calculated from the thousand seed weight (TSW).

BREEDING PERSPECTIVES

Possible breeding perspectives for improved FSU, indicated in the above review, are presented in Table 2. More research is needed to investigate which process has the highest correlation with grass seed yield, which characteristic has the largest genetic variation and which trait has the highest heritability.

It is interesting to note that several authors (Knowles and Baenziger, 1962; Ross and Adams, 1955; Lowe and Murphy, 1955; Raeber and Kalton, 1956; Nielson and Kalton, 1959; Ibrahim and Frakes, 1984; Slinkard, 1965 and Davies, 1954) reported FSU to be a fairly stable and highly heritable character. Others, (Mackay, 1960; Bean, 1969; and Bugge, 1981), mentioned large environmental effects on FSU.

A good relationship between "seed set" or "fertility index" and seed production is reported by Knowles and Baenziger (1962), Ross and Adams (1955), Nielsen and Kalton (1959), Slinkard (1965), Davies (1954), Dewey and Lu (1959) and Hearn and Holt (1969). However, the determination method of FSU influences the correlation between FSU and seed yield.

METHODOLOGY

Several studies have been made of FSU, "fertility index" or "seed set" in grasses. The results however, cannot easily be compared, because different characters appear to have been determined in various ways. Three aspects of determining FSU should be taken into account: time of determination, determination method and sampling technique.

The time of determining the number of florets for calculating the potential yield is important. If florets are counted at anthesis, the estimated potential yield is much higher than when counted after the harvest, because florets are lost during development and harvest. The time of determining the number of seeds for calculating the realized yield is also important. If seeds are counted before ripening, realized yield is much higher than when they are counted after harvest, because seeds are shed and lost during ripening, harvesting and cleaning.

Several determination methods have been described previously:

1. **Number ratio between filled and total florets.** This method has been applied quite often to uncleaned seed harvested just prior to ripeness. (Bean, 1969; Davies, 1954; Knowles and Baenziger, 1962). Empty and filled florets were separated by feeling, pressing or by examination over illuminated glass. Developing seeds are included in the seed fraction so this method gives a measure of biological FSU.

Table 2. Prospects of breeding for improved floret site utilization in grasses.

Process:	Selection for:
Pollination	- High pollen production
	- Uniform flowering (heading)
	- Lodging resistance
Fertilization and seed set	- Pollen viability
	- Ovule viability
	- Retention of pollen and ovule viability
	- Disease resistance
	- Lodging resistance
	- Compatibility
Seed development	- Disease resistance
	- Lodging resistance
	- Reduced embryo abortion
	- Uniform ripening
	- Seed retention

2. **Germination percentage.** This method is similar to method 1 in that developing, viable seeds are included. Very young seeds in the early growth stage which do not germinate are not included, however. Biological FSU measured in this way will therefore be lower than FSU as estimated by method 1 (Lewis, 1966; van Wijk, 1985).

3. **Weight ratio between cleaned and uncleaned seed.** This method is easy to apply, but does not give relevant information about FSU. After threshing, seeds are separated from empty florets and light seeds with a wind blower. Cleaned seed is much heavier than light and empty seed, so the percentage of cleaned seed is very high. This results automatically in a high, significant correlation between estimates of FSU and final seed yield (van Wijk, 1985; Raeber and Kalton, 1956).

4. **Volume ratio between cleaned and uncleaned seed.** This method gives a better estimation of the percentage of well-developed seeds than method 3 because seeds and empty florets differ less in volume than in weight (Hearn and Holt, 1969).

5. **Number ratio between cleaned and uncleaned seed.** This method is more precise than the fourth, but is very laborious. After threshing, all potential seeds are counted. Seeds are separated from empty florets and light seeds with a wind blower. Cleaned seeds are counted. Like methods 3 and 4, method 5 underestimates potential seed yield, i.e. the number of floret sites, because losses of floret sites are not included. Estimated FSU based on these methods will, therefore, be too high. Because only cleaned seeds are counted, a measure of economical FSU is given.(Lewis, 1966; Johnston, 1960).

6. **Calculated ratio between realized and potential seed yield:** FSU = yield m^{-2}/(inflorescences m^{-2} x TSW/1000 x florets/inflorescence). This method calculates economical FSU. Number of inflorescences m^{-2} and florets/inflorescence are determined at anthesis to obtain a "realistic" potential yield. FSU-values calculated in this way are much lower than values obtained with the other methods (Meijer, pers. comm.).

Method 1 seems most suitable for determining biological FSU. Shattering losses are not measured when applying these methods. For determination of biological FSU, however, shed seed should also be taken into account. For determination of economical FSU, method 6 seems the best.

The sampling technique for FSU should depend on the purpose. If the aim is to determine the FSU of a crop, inflorescences must be taken at random. If the aim is to detect genetic differences in FSU between genotypes or populations, variation due to age, developmental stage or size of the inflorescence should be reduced. Therefore, spikelets should be taken from similar positions in the infloresences and florets from the same positions in the spikelets.

SUMMARY

The seed yield of grasses needs improvement. Seed yield should become a more important selection criterion in grass breeding. The yield potential, i.e. florets m^{-2} is much higher than the realized yields. Seed yield depends largely on the degree of floret site utilization (FSU). This term is not clearly defined. A distinction should be made between biological and economical FSU. Biological FSU can be defined as the percentage of florets resulting in a viable seed. This characteristic results from several processes, i.e. pollination, fertilization, seed set and seed development. Economical FSU can be defined as the percentage of florets resulting in a harvested seed. This characteristic results from biological FSU, harvesting and cleaning.

In this paper the processes composing FSU in grasses are discussed. During each process losses may occur, resulting in decreased FSU. For several traits, genetic variation has been found which might offer perspectives when breeding for improved seed yield in grasses.

There is no agreement on heritability of FSU. However, results can hardly be compared among experiments because several definitions of FSU and various determination methods have been used. In this paper, three aspects of the methodology of FSU are discussed: time, method and sampling technique. FSU should be determined in a proper way, and methods and terms should be standardized. More research is needed on FSU in grasses to develop better selection criteria for improved seed yields.

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CHAPTER 2

Sex Plant Reprod (1989) 2:225–230

Sexual Plant Reproduction

Effects of genotype and temperature on pollen tube growth in perennial ryegrass (*Lolium perenne* L.)

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Summary. Low yield in seed crops of perennial ryegrass is related to low fertilization efficiency and low temperature during anthesis. To study the effect of genotype and temperature on pollen performance, we conducted greenhouse experiments at controlled temperatures. Individual florets of four genotypes that are known to differ in seed production were hand pollinated at four temperatures (14°, 18°, 22°, 26° C) both in vivo and via a semi-in-vitro method involving excised florets on agar. Pollen germination and tube growth were determined with UV-fluorescence microscopy and scored in six classes at 2 h after pollination in vitro and after 0.5, 2 and 5 h in vivo. In vitro, both genotype and temperature had a significant effect on the performance of self-pollen. Pollen tube growth increased with temperature. In cross-pollinations, the pistil parent had a significant effect on pollen tube growth, and there was also a significant pistil-by-temperature interaction. In vivo, genotype and temperature significantly affected pollen performance. The genotype-by-temperature interaction was only significant 5 h after pollination. One genotype with low seed yield was pseudo-self-compatible and was a relatively poor mother after cross-pollination. The effects of genotype and temperature on the growth of self-pollen might be exploited in a breeding programme.

Key words: *Lolium perenne* – Pollen tube – Genotype – Temperature – Self-incompatibility – Seed production.

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Introduction

Perennial ryegrass *Lolium perenne* L. is an important temperate grass species used for both fodder and turf. It is propagated by seed. Seed yields are rather low and unpredictable, and low seed yield results from low floret site utilization (FSU), i.e. the percentage of florets producing a seed (Griffiths 1965; Burbidge et al. 1978; Elgersma 1985). Low seed yield is related to low temperature during anthesis (Hampton and Hebblethwaite 1983), and low floret site utilization may be partly caused by low fertilization efficiency.

During the past 15 years there has been a great resurgence of interest in genetic and environmental factors that influence pollen tube growth. Recent data indicate that a large portion of the microgametophyte's genome is transcribed and translated during pollen development, germination and tube growth and that the majority of these genes are also expressed during the sporophytic stage of the life cycle (e.g. Tanksley et al. 1981; Willing and Mascarenhas 1984). Moreover, the growth rate of pollen tubes both in vitro and in vivo is determined, at least in part, by the genotype of the pollen (see Pfahler 1970; Sari-Gorla et al. 1976; Gawel and Robacker 1986; Mazer 1987). Various environmental conditions, such as temperature, humidity and salinity (Zamir et al. 1981; Sacher et al. 1983) are also known to influence germination and pollen tube growth rates. Consequently, as one part of a comprehensive research programme examining genetic variation for seed yield components in perennial ryegrass, we investigated the effects of temperature and genotype on the performance of pollen following self- and cross-pollination.

Materials and methods

Materials

Four diploid *Lolium perenne* genotypes which differ in seed yields (as observed in 1986 on spaced plants in clonal replications in field trials near Wageningen, The Netherlands) were chosen for this study. Two genotypes, 1–33 and 5–7, had a high average seed yield of 78.4 and 95.3 g per plant, respectively, and 0.24 and 0.25 g per spike, respectively. Genotype 1–11 had an intermediate seed yield (26.1 g per plant and 0.12 g per spike), and genotype 1–50 had a low seed yield per plant (13.5 g) and per spike (0.04 g). Seed yield per spike is an indication of floret site utilization. Genotypes 1–11, 1–33 and 1–50 originate from cv 'Semperweide' and genotype 5–7 originates from cv 'Perma'.

In the autumn of 1986 the four genotypes were cloned, transplanted into 32 pots and vernalized in a greenhouse at 5° to 8° C during the winter. After vernalization eight pots of each genotype were placed into each of four greenhouses on March 25, 1987, and grown at a constant temperature of 26°, 22°, 18° or 14° C. Flowering began in early May in the 26° C greenhouse, followed in June by the clones in the 22°, 18° and 14° C greenhouses. Devernalization occurred in various plants, especially at high temperatures. At 26° C the plants were rather small and had tiny spikes; at 18° and 14° C, the plants were the most vigorous.

Pollinations in vitro

Within each greenhouse semi-in-vitro pollinations were performed among the four selected genotypes. We shall refer to these as pollinations in vitro in this paper. For each temperature, two or more ovaries with pistils of each of the four genotypes were excised and transferred to an assigned position on agar in each of five petri dishes. Because anthesis occurs in the mid-morning, the pistils were transferred in the late afternoon of the day before anthesis. The five dishes were stored overnight in the same greenhouse as the one from which the pistils were obtained. The next morning, in each greenhouse, pollinations were made by shaking the spikes of the pollen parent so that a cloud of fresh pollen was released from one of the genotypes over an open dish containing the pistils of the four genotypes. Each genotype was used to pollinate one dish. The fifth dish was opened, but no pollen was released over it. This resulted in the equivalent of a complete diallel cross and a sham (control) pollination. The five dishes were incubated in the greenhouse, and after 2 h the pistils were fixed in FAA and stored at 4° C in 15 *M* ethanol. The entire experiment was repeated at least twice at each temperature. One of the genotypes (1–50), however, was not included in the experiments at 22° C, because of insufficient flowering when the other genotypes were in bloom. At 26° C, we pollinated on May 21 and 26 and on June 25; at 22° C, on June 1, 2 and 25; at 18° C, on June 10, 17, 22 and 25; and at 14° C, on June 22, 23 and 25.

Pollinations in vivo

In vivo the four selected genotypes were used as female parents and will be referred to as mothers. Forty unselected genotypes from different perennial ryegrass varieties were used as pollen parents, and these will subsequently be referred to as fathers. These plants were vernalized as described in the Materials, but were all placed at 22° C on March 25 in order to provide pollen of the same quality for pollinations at different temperatures. Individual florets of the four mothers were pollinated immediately after the opening of the glumes and emergence of the stigma. Spikelets were numbered from the bottom of the spike upwards, and within spikelets, florets were numbered from the base of the spikelet upwards. In this manner, floret position within a spike was determined. Pollinations were made by releasing a cloud of fresh pollen from the fathers over the opened florets of the mother. At least five fathers were taken at random from among the forty genotypes and transferred from 22° C to houses containing the mothers shortly before flowering of the mothers. In this way open pollination with a large quantity of compatible pollen was realized. After pollination the fathers were put back into the greenhouse kept at 22° C. Florets were fixed at 0.5, 2 and 5 h after pollination. Whole spikelets were collected, fixed in FAA and stored at 4° C in 15 *M* ethanol. The hand-pollinated florets were dissected from the spikelets using a binocular microscope.

At 26° C pollinations were conducted on May 19–22, 27 and 31; at 22° C, on May 21, 26, 27 and on June 11 and 24; at 18° C, on June 10, 11 and 24; and at 14° C, on June 23–25, 27 and on July 2 and 3.

Observations

Fixed ovaries with pistils were rinsed in water and stained with 1% aniline blue, mounted, gently squashed and examined by fluorescent microscopy. Two scores were recorded for each stigma: the performance of a random sample of 25 pollen grains and that of the three most vigorous pollen tubes. Stigmas that were damaged during dissection or during the staining and mounting procedures were not scored. One score, averaged over both stigmas, was calculated per floret for mean pollen performance (meanpp) and one for maximum pollen performance (maxpp).

The following scale was used: 0 = no germination; 1 = germination but no penetration of the sigma by the pollen tubes; 2 = penetration of the stigmatic hairs, but the pollen tubes have not advanced to the main branch (stylar canal) of the stigma; 3 = growth of the pollen tube into the main branch of the stigma; 4 = pollen tubes have advanced to the base of the stigma; 5 = pollen tubes have entered the ovary; 6 = pollen tubes have reached the micropyle. The date was used as a covariable within each level of temperature, while the factors temperature, father and mother were considered fixed.

Results

Pollinations in vitro

No pollen was deposited on the vast majority of the stigmas in the sham pollination dishes. On four of the sham pollinated stigmas, three to ten non-germinated pollen grains were observed, while one stigma had two germinated grains (scores = 1 and 2) and another stigma had one germinated grain (score = 1). These sham pollinations reveal that we had indeed selected virgin pistils and that pollen contamination was not a problem. In total, 306 florets were scored, and on most florets both stigmas could be observed. The data on meanpp and maxpp are presented in Tables 1 and 2.

Within pistils of the same genotype, pollen tubes had generally advanced further after cross-

Table 1. Mean pollen performance (Meanpp) ±standard error in four perennial ryegrass genotypes 2 h after pollination in vitro at four temperatures (T). Self-pollinations are underlined. For explanation of codes see text

T (°C)	Mother	Father			
		5–7	1–11	1–33	1–50
14	5–7	0.9±0.1	2.7±0.1	2.3±0.2	2.0±0.0
	1–11	2.0±0.0	0.8±0.2	2.6±0.1	1.8±0.2
	1–33	2.6±0.4	1.9±0.4	1.4±0.2	3.1±0.2
	1–50	2.2±0.2	1.8±0.2	2.3±0.2	1.5±0.1
18	5–7	1.4±0.1	2.9±0.3	3.1±0.3	3.3±0.1
	1–11	2.9±0.3	1.7±0.2	3.1±0.1	2.7±0.3
	1–33	3.3±0.3	2.8±0.4	1.7±0.2	2.6±0.2
	1–50	2.3±0.1	2.3±0.2	2.5±0.3	2.4±0.2
22	5–7	1.7±0.1	2.6±0.2	3.0±0.3	–
	1–11	3.2±0.3	1.5±0.2	3.4±0.2	–
	1–33	2.6±0.2	2.3±0.2	1.9±0.3	–
	1–50	–	–	–	–
26	5–7	1.4±0.4	3.6±0.2	3.3±0.2	3.7±0.3
	1–11	3.4±0.3	1.4±0.4	3.3±0.1	3.0±0.1
	1–33	3.1±0.6	2.5±0.2	2.1±0.3	3.9±0.1
	1–50	3.2±0.2	3.0±0.6	3.0±0.1	2.8±0.1

Table 2. Maximum pollen performance (Maxpp) ±standard error in four perennial ryegrass genotypes 2 h after pollination in vitro at four temperatures (T). Self-pollinations are underlined

T (°C)	Mother	Father			
		5–7	1–11	1–33	1–50
14	5–7	1.4±0.1	3.3±0.1	3.2±0.2	3.0±0.3
	1–11	2.6±0.4	1.6±0.3	3.2±0.2	2.7±0.5
	1–33	3.1±0.1	2.7±0.4	1.8±0.2	4.2±0.1
	1–50	3.0±0.0	2.7±0.2	3.5±0.3	2.3±0.2
18	5–7	2.0±0.2	3.8±0.4	3.8±0.4	4.2±0.2
	1–11	3.8±0.5	2.6±0.2	3.8±0.1	3.5±0.2
	1–33	4.0±0.5	3.8±0.5	2.8±0.3	3.3±0.2
	1–50	3.1±0.1	3.4±0.2	3.9±0.3	3.3±0.3
22	5–7	2.6±0.1	4.1±0.2	4.1±0.5	–
	1–11	4.5±0.2	2.4±0.1	4.6±0.2	–
	1–33	3.6±0.2	3.3±0.3	2.9±0.4	–
	1–50	–	–	–	–
26	5–7	2.4±0.4	4.8±0.1	4.7±0.3	5.0±0.0
	1–11	4.1±0.4	2.4±0.4	4.5±0.2	3.7±0.1
	1–33	4.2±0.7	3.6±0.3	3.0±0.2	4.7±0.3
	1–50	3.3±0.3	3.7±0.7	4.1±0.2	3.6±0.1

pollination than after self-pollination at all temperatures. This was expected, since perennial ryegrass is a self -incompatible species (Spoor 1976). Therefore, we separately analysed the data on pollen performance after self- and cross-pollinations.

Among the self-pollinations, significant genotypic effects were present for both meanpp and

Table 3. Summary of the analyses of variance for the performance of self-pollen in vitro; * $P \leq 0.05$; ** $P \leq 0.01$

Source	*df*	Meanpp		Maxpp	
		MS	F	MS	F
Temperature (T)	3	1.17	3.55*	2.17	6.01*
Genotype (G)	3	4.49	13.64**	4.96	13.75**
T–G	8	0.38	1.14 ns	0.32	0.89 ns
Residual	101	0.33		0.36	

Table 4. Summary of the analyses of variance for the performance of cross-pollen in vitro. ns, Not significant; * $P \leq 0.05$

Source	*df*	Meanpp		Maxpp	
		MS	F	MS	F
Temperature (T)	2	0.57	1.97 ns	0.04	0.08 ns
Mother (M)	3	2.23	7.65*	2.54	5.12*
Father (F)	3	1.04	3.57*	1.19	2.40 ns
M–T	8	0.59	2.02*	1.31	2.65*
F–T	8	0.20	0.67 ns	0.28	0.57 ns
M–F	5	0.65	2.23 ns	0.93	1.87 ns
M–F–T	11	0.51	1.74 ns	0.52	1.05 ns
Residual	137	0.29		0.49	

for maxpp (Table 3). In general the pollen tubes of genotypes 1–33 and 1–50 advanced further than those of genotypes 5–7 and 1–11 (Tables 1 and 2). Because the four genotypes flowered on different dates in the 14°, 18°, 22° and 26° C greenhouses, it was usually not possible to perform the experiments at different temperatures on the same day; thus date was introduced as a covariable in the model. After elimination of the effect of pollination date within temperature, temperature was observed to have a significant (Table 3) and positive (Tables 1 and 2) effect on pollen performance. The interaction of genotype by temperature was not significant (Table 3).

The results of the analyses of the cross-pollinations are summarized in Table 4. Temperature affected neither meanpp nor maxpp. The effect of the mother on both meanpp and maxpp was significant. The effect of the father on meanpp was significant, but on maxpp it was not. The mother-by-temperature interaction was significant for both meanpp and maxpp. That is, the performance of the cross-pollen on some maternal genotypes (e.g. 1–33) varied only slightly with temperature, while on other genotypes (e.g. 5–7) temperature had a pronounced effect. Maternal-by-paternal interaction, father-by-temperature interaction and the three-way father-by-mother-by-temperature interaction were not significant.

Pollinations in vivo

In florets fixed 0.5 h after pollination, rare pistils contained pollen tubes that had already reached the micropyle, presumably due to pollen contamination prior to the artificial pollination since the majority of the pollen tubes had not yet penetrated the stigma. However, this was only found at 26° C, twice in genotype 1–33 and once in genotype 1–11, and at 22° C, once in genotype 5–7 and once in genotype 1–50. Therefore, we conclude that pollen contamination was not a serious problem. These florets were disregarded in the analyses, which left 207 florets to be studied at 0.5 h and after pollination, 216 florets at 2 h after pollination and 267 florets at 5 h after pollination. The data on maxpp are presented in Table 5. Only the most vigorous pollen grains had germinated at 0.5 h after cross-pollination, but their tubes generally had not penetrated the stigmatic hairs. At 2 h after pollination the three most virgorous pollen tubes had entered the stigmas and had sometimes advanced to the base of the stigmas. After 5 h they were observed in the main branch of the stigmas and had sometimes already penetrated into the ovary (Table 5).

The results of the analyses of variance within each of the three sampling times are summarized in Table 6. The analysis of variance of meanpp is not presented because the results were similar to those of maxpp.

At 0.5, 2 and 5 h after pollination, significant effects of temperature and genotype on maxpp were found (Table 6). Pollen performance tended to increase with temperature (Table 5). The genotype-by-temperature interaction was only significant at 5 h after pollination (Table 6).

Table 5. Maximum pollen performance (Maxpp) ± standard error in four perennial ryegrass genotypes 0.5, 2 and 5 h after open pollination in vivo at four temperatures (T)

T (°C)	Mother	Hours after pollination		
		0.5	2	5
14	5– 7	0.50 ± 0.18	–	3.75 ± 0.64
	1–11	1.31 ± 0.26	2.33 ± 0.32	2.79 ± 0.31
	1–33	1.41 ± 0.13	3.36 ± 0.33	2.24 ± 0.34
	1–50	1.27 ± 0.22	–	–
18	5– 7	0.91 ± 0.22	1.90 ± 0.28	2.20 ± 0.39
	1–11	1.98 ± 0.18	3.06 ± 0.31	3.45 ± 0.32
	1–33	1.55 ± 0.12	2.52 ± 0.32	3.95 ± 0.34
	1–50	1.77 ± 0.20	2.95 ± 0.51	5.61 ± 0.29
22	5– 7	2.19 ± 0.31	4.00 ± 0.41	5.12 ± 0.25
	1–11	0.95 ± 0.09	2.95 ± 0.35	4.00 ± 0.53
	1–33	2.00 ± 0.67	4.47 ± 0.41	4.73 ± 0.27
	1–50	–	–	3.33 ± 0.88
26	5– 7	1.33 ± 0.23	3.47 ± 0.40	3.07 ± 0.27
	1–11	1.89 ± 0.20	4.25 ± 0.25	4.63 ± 0.30
	1–33	1.11 ± 0.13	4.73 ± 0.21	4.36 ± 0.45
	1–50	–	–	–

Discussion

Our data show that an environmental factor (temperature) as well as the genotype of the mother plant affect the performance of both self- and cross-pollen. As expected, temperature affected the performance of cross-pollen in vivo and the performance of self-pollen in vitro, but no significant effect of temperature on cross-pollen was observed in vitro. This was probably due to increased variance resulting from variation in pollination time. However, the mother-by-temperature interaction was significant (Table 4).

Cross-pollen outperformed self-pollen because perennial ryegrasss has a gametophytic self-incompatibility system (Spoor 1976; Cornish et al. 1979; McGraw and Spoor 1983). The resulting self-incompatibility is strong, but not complete, and small quantities of seed may be obtained when individual plants are self-pollinated (Jenkins 1931; Foster and Wright 1970; Cornish et al. 1980). This behaviour is usually referred to as pseudo-self-compatibility and individual plants differ with re-

Table 6. Summary of the analyses of variance for pollen performance (Maxpp) of cross-pollen in vivo at 0.5, 2 and 5 h after pollination. ns, Not significant; * $P \leq 0.05$; ** $P \leq 0.01$

Source	Hours after pollination								
	0.5			2			5		
	df	MS	F	*df*	MS	F	*df*	MS	F
Temperature (T)	3	2.94	3.48*	3	8.49	3.56*	3	37.86	15.86**
Genotype (G)	3	6.07	7.20**	3	16.75	7.03**	3	21.97	9.20**
T–G	4	2.06	2.34 ns	2	0.79	0.33 ns	4	18.67	7.82**
Residual	255	0.84		203	2.38		252	2.39	

spect to their pseudo-self-compatibility (Fearon et al. 1983; Winkelhorst and den Nijs, unpublished work). Moreover, year-to-year variation in seed set of some genotypes after self-pollination indicates that environmental factors may also influence the degree of pseudo-self-compatibility (Jenkins 1931; Beddows et al. 1962).

In perennial ryegrass a callose reaction occurs after the contact of an incompatible pollen tube with the stigma and this prevents further pollen tube growth (Lundqvist 1961). Following selfing in vitro, the stigmas generally had not been penetrated in genotypes 5–7 and 1–11 (Table 1). In genotype 1–33, however, most pollen tubes had penetrated the stigmatic hairs at 26° C, and in genotype 1–50, at 18°, 22° and 26° C, indicating that, in these genotypes, self-incompatibility was less strong. The three most vigorous pollen tubes entered the stigma in all genotypes at 18°, 22° and 26° C; in genotype 1–50 penetration even occurred at 14° C. So it is obvious that both genotype and temperature influenced the growth of self-pollen. In a grass breeding programme, these effects might be exploited. For example, some genotypes might best be self-pollinated at a high temperature.

Perhaps environmental effects, such as temperature, are responsible for some of the confusion concerning the number of loci involved in the incompatibility system of perennial ryegrass (Lawrence et al. 1983; McGraw and Spoor 1983; Leach 1984). We found that the performance of self-pollen at higher temperatures may exceed the performance of cross-pollen at a lower temperature. Consequently, unless temperature is carefully controlled, day-to-day fluctuations in temperature could obscure the degree of self-incompatibility.

There are some interesting contrasts among the four genotypes with respect to the performance of their pollen and pistils in self- and cross-pollinations in vitro. This is illustrated in Fig. 1 (the data are extracted from Table 2). The performance of self-pollen follows a pattern similar to the performance of pollen on stigmas of other genotypes. In contrast, the receptivity of the stigma seems to follow a different pattern. In essence, genotype 1–50, which is pseudo-self-compatible, is an outstanding father but a relatively poor mother (Fig. 1). On the other hand, the strongly self-incompatible genotype 5–7 is a less good father and a relatively good mother. Perhaps strong self-incompatibility might actually promote the growth of cross-pollen while inhibiting the growth of self-pollen. Clearly, a larger study with more genotypes would be needed to explore such possibilities.

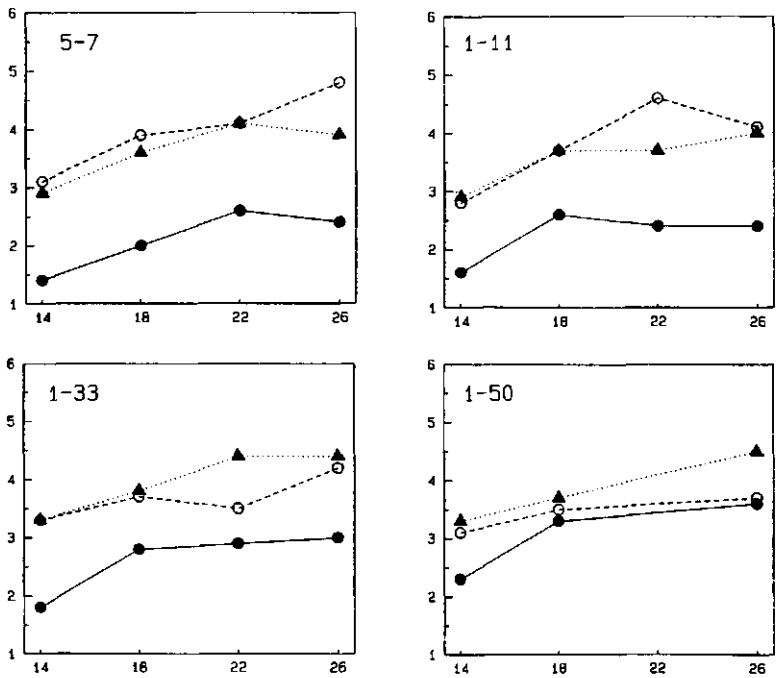

Fig. 1. Maximum pollen performance (maxpp; *ordinate*) of pollen of four perennial ryegrass genotypes in their own stigmas (● behaviour after selfing) and in other stigmas (▲ behaviour as a father) and maxpp of cross-pollen on the stigmas of these four genotypes (○ behaviour as a mother) at four temperatures (°C; *abscissa*) in vitro. Data were extracted from Table 2

The poor performance of cross-pollen on pistils of genotype 1–50 may explain the low seed yield of this genotype. On the other hand, pistils of genotype 5–7 permit rapid growth of cross-pollen, and this genotype has a high yield. In genotypes 1–11 and 1–33 the relationship between behaviour as a mother and seed yield is less clear.

Obviously, pollination in vitro provides technical advantages over pollination in vivo. In vitro, emasculation is easy and time of pollination can be adjusted to the pollen source. In vivo, simultaneous flowering of both parents is difficult to achieve, as time of flowering during the morning depends on genotype and temperature. In vivo, some self-pollen is always shed onto the stigmas while one is hand-pollinating with cross-pollen, unless the mother is male sterile or has been emasculated. If for some reason no adequate cross-pollination occurs, the pollen observed will be mainly self-pollen. But even after abundant pollination with viable, compatible cross-pollen, the stigma always contains a mixture of cross- and self-pollen. Therefore, the performance of the most vigorous pollen tubes will give a better indication of the performance of cross-pollen than the average pollen performance.

In the in-vitro experiments pollen was produced inside the greenhouse where the pollinations were conducted, and therefore the pollen may have a different quality at each temperature. In the in-vivo experiments all the pollen was produced at 22° C, therefore performance of the three most vigorous pollen tubes in vitro and in vivo can strictly only be compared after 2 h at 22° C. However, the pollinations were conducted on different days, and since date had a pronounced effect on pollen performance, only a rough comparison is possible between our results obtained in vitro and in vivo. A detailed study with more genotypes is necessary to compare both methods. However, the trend that the genotype of the mother affects both mean and maximum pollen performance was the same in both our experiments in vitro and in vivo.

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CHAPTER 3

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Cytology of seed development related to floret position in perennial ryegrass (*Lolium perenne* L.)

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Summary

In seed crops of perennial ryegrass, *Lolium perenne* L., yields are low and only 15 to 20% of the florets produce a harvestable seed. This study was conducted to determine if seed abortion was a major cause of low floret site utilization (FSU) and therefore of low seed yield in spaced plants. Spikes of four spaced-planted genotypes of perennial ryegrass were harvested and fixed at one-week intervals one to five weeks after the beginning of anthesis. Of a total of 74 spikes, over 13,000 florets were examined with a binocular microscope and 66 whole spikelets were microtome sectioned. The histology of the pro-embryo is presented in relation to the development of the endosperm. In one low yielding, two intermediate and one high yielding genotype, biological FSU was 8, 72, 73 and 63%, respectively. In all genotypes, 90% of the unproductive florets showed ovule degeneration within a few days after flowering. A few florets were sterile or contained a degenerated embryo sac already before flowering and about 5% of the unproductive florets showed seed abortion later than one week after flowering. Within a spikelet, successful fertilization did not decline from the basal to the distal floret. Although in later stages distal florets had slightly less seeds, unproductive florets were found at all floret positions. Biological FSU decreased mainly by ovule degeneration shortly after flowering. In spaced plants, seed abortion was not important to low seed yield.

Introduction

Perennial ryegrass, *Lolium perenne* L., is an important temperate grass species. It is propagated by seed, but seed yields are rather low and unpredictable. New cultivars can only be commercialized, however, if the seed production is satisfactory. Therefore, seed yield should become a more important selection criterion in grass breeding (Griffiths, 1965; Elgersma, 1985).

Seed crops of perennial ryegrass have a high yield potential, but realized yields are low (Griffiths et al., 1973; Hebblethwaite, 1977). Only 15 to 20% of the available florets produce a harvestable seed. The reasons for this very low economical floret site utilization (FSU) are many: unproductive florets may be sterile (Knowles & Baenziger, 1962) or unpollinated, which is usually attributed to unfavorable weather during anthesis. Florets may be pollinated but not fertilized, either due to incompatibility or to poor weather conditions for pollen germination or tube growth. Florets may become fertilized, but embryos or developing seeds may abort (Burbidge et al., 1978; Hill, 1980). Seeds may shatter before harvest (Anslow, 1964; Elgersma et al., 1988; Stoddart, 1964) or be lost

during harvesting and cleaning (Meijer, 1985). Finally, factors such as seed crop management, lodging, uneven ripening and diseases also affect seed yield (Vyncke, 1981).

FSU is therefore a complex trait and estimates depend upon the developmental stage, the sampling technique and the determination method. Biological FSU, defined as the percentage of initial florets producing a viable seed, results from pollination, fertilization and seed development. Economical FSU, defined as the percentage of initial florets contributing to the harvested seed, is determined by shattering, harvesting and cleaning as well (Elgersma, 1985).

Detailed observations are necessary for a better understanding of the problems associated with low seed production in perennial ryegrass (Marshall, 1985). Developmental morphology of the embryo is described in cereals (Bhatnagar & Chandra, 1975; Suetsugu, 1951; 1953), but in forage grasses little is known about seed development and abortion. Reusch (1959) observed the early growth rates of the endosperm and the embryo of perennial ryegrass.

Not much is known about the position of unproductive florets within the inflorescence. Anslow (1963; 1964) and Burbidge et al. (1978) mentioned a decline in successful fertilization within a spikelet from the basal to the distal floret. They also found that embryos of distal florets were more susceptible to abortion. However, Knowles & Baenziger (1962) observed that although upper florets within spikelets generally produced less seeds than lower florets, unproductive florets were found throughout spikelets and were not confined to distal florets.

In this study seed development, degeneration of ovules, and seed abortion were studied cytologically in four genotypes of perennial ryegrass to investigate differences in biological FSU. The percentage productive florets was related to genotype, floret position and developmental stage.

The fruit, consisting of a single seed fused to the pericarp, is botanically a caryopsis but will be referred to as a seed in this paper.

Materials and methods

Four unrelated diploid genotypes of perennial ryegrass with contrasting seed production were used that had been examined during several years (Table 1). Two genotypes originate from cultivars, two from breeding families. The extremely low yielding genotype 1–5 descends from a cross of perennial ryegrass with Westerwold ryegrass; the progeny was backcrossed three times with perennial ryegrass. The other genotypes combined a fair to high seed yield with good seed retention (Elgersma, unpublished results).

In 1985, the genotypes were grown on a sandy soil in large spaced planted nursery at Wolfheze, the Netherlands. The plants were not tied up and spikes lodged after anthesis. The plants were open-pollinated with compatible pollen. Flowering was defined as anther emergence of any individual floret, regardless of position or time of opening on a plant. Anthesis was defined as the first floret to flower on a spike. In each genotype, four individually labeled spikes were collected at random at one-week intervals starting at one week after anthesis (WAA) until five WAA. Whole spikes were fixed in FAA (900 ml 15 M ethanol, 50 ml acetic acid, 50 ml formaldehyde l^{-1}) and stored at 4° C in 15 M ethanol. The spikes were dissected and spikelets were observed with a binocular microscope. Florets were checked for the presence of a seed and the size of the seed was determined. Rudimentary florets at the tip of each spikelet were omitted.

Sixty-six spikelets containing 587 florets were chosen for cytological and histological investigation (Table 2). They were dehydrated in ethanol

Table 1. Seed yield of spaced plants of perennial ryegrass, determined during four years

Genotype	Source	Yield per plant (g)			
		1982	1983	1985	1986
1–5	175–5	–	–	1.1	–
10–3	'Perma'	11.2	17.0	10.1	7.5
10–7	'Lamora'	10.2	14.4	9.7	7.3
5–8	371–9	20.9	18.9	17.9	34.4

and embedded in paraffin. Whole spikelets were sectioned at a thickness of 12 μm along their axis. The sections were stained with safranin and fast green and examined with a light microscope.

For each genotype, the numbers of spikelets, florets and seeds per spike were determined and the percentage of productive florets was calculated. This percentage was also related to floret positon within the spike. Therefore, florets were numbered from the base of a spikelet upwards. Per spike, florets at positions 1, 2 and 3 were grouped, florets at positions 4 to 6 were grouped, florets at positions 7 to 9 were grouped, etcetera.

Results

Normal development

The inflorescence of perennial ryegrass is a spike, generally containing 20 to 30 spikelets (Fig. 1). Each spikelet contains 5 to 13 normally developed florets with a rudimentary floret at the top. Basal and intermediate spikelets have more florets than apical spikelets. A floret consists of a lemma and a palea enclosing two lodicules, one ovary with two stigmas and three anthers. The ovule is anatropous and bitegmic. The mature embryo sac is of the *Polygonum* type and consists of an egg cell, and two small synergids located at the micropylar end, a central cell, and three prominent antipodals at the chalazal end.

Table 2. Numbers of sectioned spikelets (s) and florets (f) studied in four genotypes of perennial ryegrass at four stages of the sampled spikes

Genotype	s/f	Stage (WAA)			
		1	2	3	4
1–5	s	6	18	–	–
	f	53	163		
10–3	s	4	6	2	–
	f	40	50	20	
10–7	s	3	10	–	3
	f	25	63		23
5–8	s	4	8	2	–
	f	49	78	23	

In spikes of perennial ryegrass, flowering generally starts in the central spikelets. It continues along the spike rather rapidly to the top and more slowly to the base. Within a spikelet, flowering proceeds acropetally. Under favorable weather conditions, more than one floret within a spikelet flowers during the same day. At flowering, the lodicules enlarge and the glumes open. Stamens and stigmas emerge simultaneously from the glumes. Pollen is released within a few minutes after anther emergence. The time between pollination and fertilization depends upon both genetical and environmental factors. In perennial ryegrass, pollen germinated within 0.5 hour, and 2 to 5 hours after pollination pollen tubes had reached the micropyle at 14 to 26° C (Elgersma and Stephenson, unpublished results). Reusch (1959) observed divisions in endosperm nuclei 12 hours after pollination.

Prior to flowering, the ovary is round and has an average diameter of 0.5 mm (Fig. 2). In florets with recently fertilized embryo sacs the ovule enlarges and the ovary increases in length as well. Within the embryo sac, the zygote is located at the micropylar end and synergids are no longer visible. We were not able to observe synergid degeneration after pollination. Shortly after fertilization, the primary endosperm nucleus divides repeatedly and free-nuclear endosperm is formed. Nuclei are scattered mainly near the zygote, around the antipodals, and in a thin layer of cytoplasm lining the periphery of the embryo sac. The antipodals enlarge. Due to the growth of the endosperm, the antipodals become located in a lateral position towards the adaxial side (Fig. 3).

Some days after flowering, when the ovary has elongated to 1.5 mm, the zygote divides, by forming a transverse wall, into an apical cell at the chalazal end and a basal cell at the micropylar end. This pro-embryo is surrounded by a thick layer of free-nuclear endosperm. Then the endosperm cellularizes. Cell formation starts around the embryo and proceeds to the chalazal end of the ovary. The antipodals persist and enlarge further (Fig. 4). As cellularization of the endosperm continues, the first layer of endosperm cells at the chalazal end forms along the periphery of the embryo sac. The

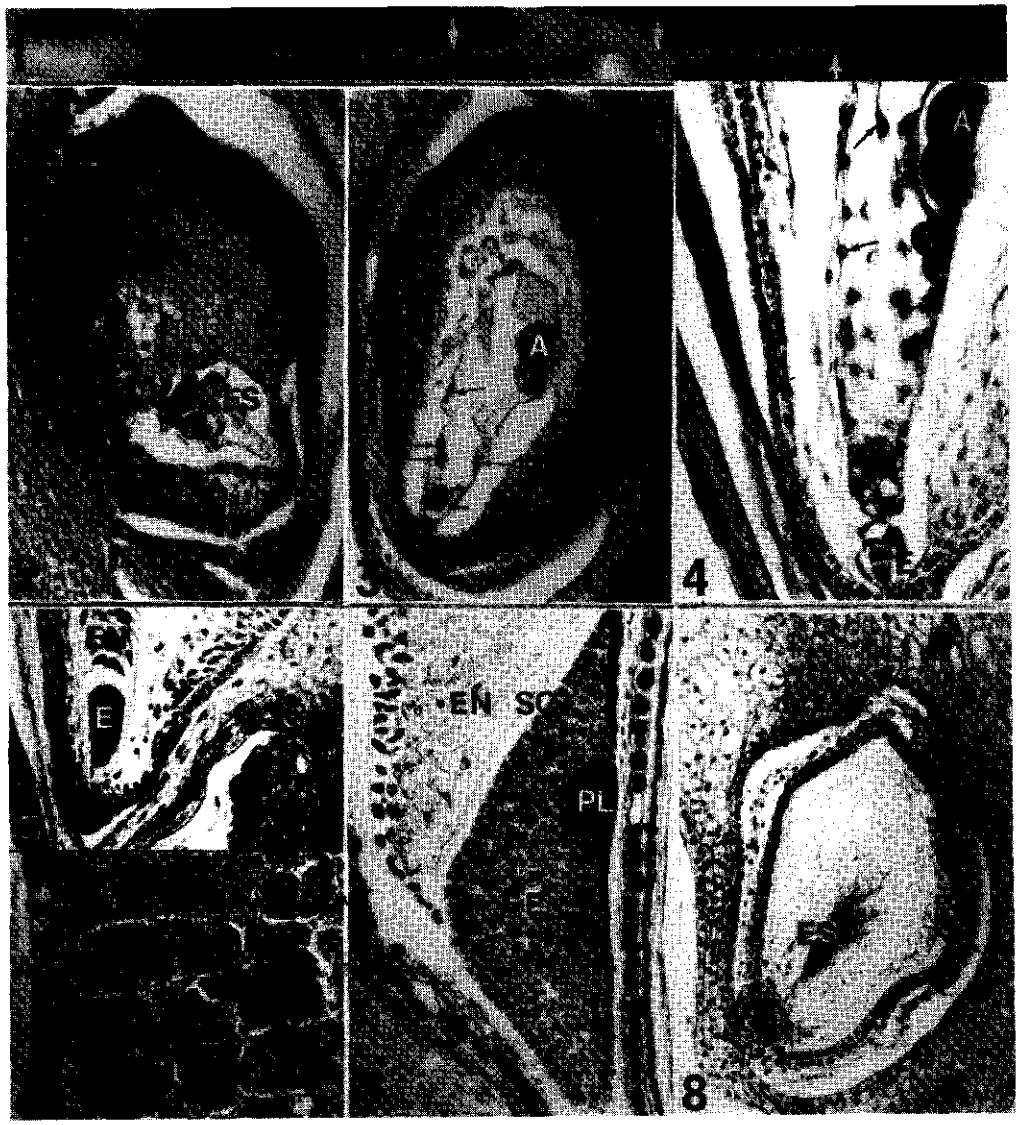

Fig. 1. Spike of *Lolium perenne* L., containing 26 spikelets. Counted from the base, the fifth, thirteenth and twenty-second spikelet have been removed (arrows) (× 0.5).
Fig. 2. Mature ovule with embryo sac (ES); the central cell (C) is visible (× 120).
Fig. 3. Ovule with zygote (Z), free-nuclear endosperm (arrows), and two antipodals (A) visible (× 120).
Fig. 4. Ovule with two-celled proembryo (E), as the cellularization of endosperm (EN) starts. Free-nuclear endosperm is indicated by arrows. Two of the three antipodals (A) are prominently visible (× 120).
Fig. 5. Part of ovule with globular embryo (E) and cellular endosperm (EN) (× 120).
Fig. 6. Detail of endosperm in ripe seed, cells contain starch. AL is aleuron layer (× 190).
Fig. 7. Differentiated embryo (E), scutellum (SC) and plumule (PL) are visible. EN is endosperm (× 120).
Fig. 8. Ovule with collapsed embryo sac (ES) in pre-anthesis stage (× 120).

apical cell of the pro-embryo divides perpendicularly to the plane of the first division. After the three-celled stage, division takes place in the two upper cells in a plane parallel to the first division, resulting in a five-celled pro-embryo. After this stage, the pro-embryo develops further into a multi-cellular, pear-shaped embryo. The endosperm continues cellularizing and the antipodals decline. When the cellularization of the endosperm is completed and the central part of the embryo sac is filled with cells, the embryo is globular (Fig. 5).

Two weeks after fertilization, embryo differentiation has begun and starch is found in the endosperm cells. The outer layer of the endosperm consists of one layer of smaller cells without starch, which become the aleuron layer (Fig 6). In ripening seed, differentiation of the provascular and meristematic tissue is observed (Fig. 7). In a fully differentiated embryo, a scutellum, plumule, and radicle are visible.

In spikes fixed one week after anthesis (one WAA), the developmental stages of the florets ranged from globular embryos with cellularized endosperm at the base of the central spikelets to immature embryo sacs at the upper parts of apical and basal spikelets. At two WAA, developing seeds at the base of the central spikelets contained differentiating embryos and endosperm cells with starch. In the upper parts of the central spikelets and in the lower parts of the other spikelets, seeds with free-nuclear endosperm were found. At this time, some top florets of the basal spikelets had not flowered yet. Three WAA all normally developed florets had flowered and four WAA seeds of central and apical spikelets were mature. Distal seeds started to shatter from the upper spikelets. By five WAA many seeds had already been shed and the remaining seeds were completely ripe, with solid, granular endosperm.

Ovule degeneration and seed abortion

Many florets did not set seed or exhibited arrested seed development of various types.

In some non-apical florets, the ovary was rudimentary and non-functional reduced anthers were present inside the glumes. Apparently, these florets were completely sterile. In all four genotypes, they occurred in very low frequencies.

In a low percentage of the sectioned florets, degeneration started before anthesis and affected the embryo sac, which collapsed (Fig. 8). The ovaries were similar in size to normal ovaries at the same developmental stage.

In about 90% of the unproductive florets, degeneration started within a few days after flowering and affected the nucellar tissue. This was similar in all genotypes. Some nucellar cells were swollen and stained more intensely with safranin than normal cells (Fig. 9). In more advanced stages of degeneration, the contents of the cells of the ovule disappeared (Fig. 10).

In about five percent of the unproductive florets, seeds degenerated in later stages. The endosperm in such aborting seeds was in the early cellular stage and the embryo was globular. The embryo shrivelled (Fig. 11) and stained very darkly. In the endosperm, the content of cells was hydrolyzed and whole cells deteriorated. Such aborting seeds were easily distinguishable under a binocular microscope. In a few florets with collapsed seed, insects were found inside the glumes.

Summarizing, we found that the majority of the unproductive florets showed ovule degeneration shortly after flowering in all genotypes. No differences among genotypes occurred for the percentage of sterile florets or aborting seeds.

Floret site utilization related to genotype

The total number of examined spikes and the average number of spikelets per spike of the four genotypes are summarized in Table 3. Genotypes 10–7 and 5–8 had more spikelets per spike than genotypes 1–5 and 10–3.

The number of florets per spikelet and per spike, the percentage of florets at pre-flowering stages and the percentage of spikelets with one or more shattered seeds are presented in Table 4. Genotypes 1–5 and 5–8 initially had a high number of florets per spikelet, compared to genotypes 10–3 and 10–7. Not unexpectedly (Table 3), rather large differences occurred for the average number of florets per spike. At one WAA, in all genotypes many florets (9 to 26%) were still in pre-flowering stages.

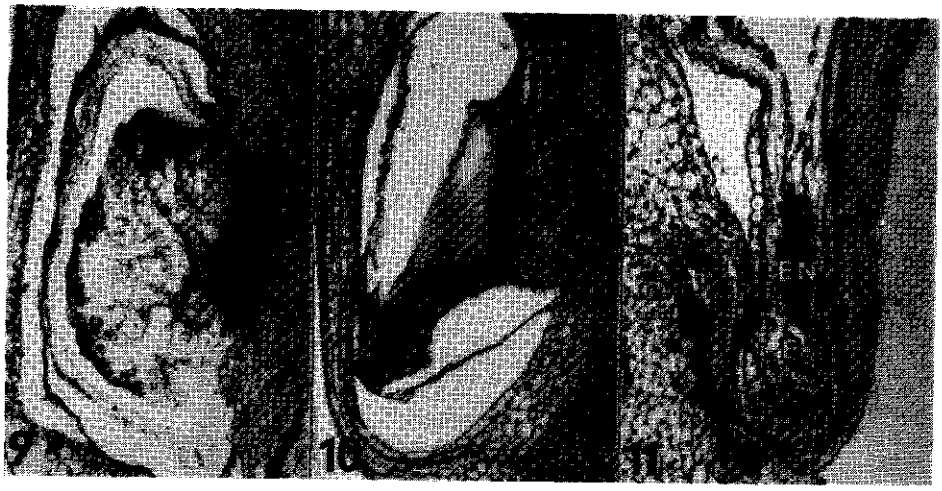

Fig. 9. Ovule degeneration just after pollination (× 120).
Fig. 10. Ovule degeneration as in Fig. 9, but in a more advanced stage of degeneration (× 120).
Fig. 11. Late abortion, resulting in a degenerated ovule and ovary. E is embryo, EN is cellular endosperm (× 120).

The number of spikelets per spike (Table 3) is independent of age, but the number of florets per spikelet and per spike declined at four and five WAA as a result of shattering (Table 4).

Table 5 shows the number of normally developing seeds and the percentages of both actual and initial florets containing a seed. In all genotypes, the actual percentage of productive florets remained fairly stable with age, but the absolute numbers of florets (Table 4) and seeds (Table 5) per spike decreased in spikes sampled one to five WAA due to shattering. This decline was especially observed in genotypes 10–7 and 5–8, where at five

Table 3. The number of examined spikes (n) and the average number of spikelets per spike with standard error (SE) and range in four genotypes of perennial ryegrass

Genotype	n	Number of spikelets per spike	
		mean ± SE	range
1–5	18	23.7 ± 0.3	21–26
10–3	17	22.3 ± 0.5	19–28
10–7	19	25.8 ± 0.4	22–28
5–8	20	26.2 ± 0.6	23–31

Table 4. The average number of florets per spikelet and per spike, the percentage of florets at pre-flowering stages (pre) and the percentage of spikelets with shattered seeds (shat) of four genotypes of perennial ryegrass at five stages

Genotype	Stage (WAA)	Number of florets /spikelet	Number of florets /spike	pre (%)	shat (%)
1–5	1	9.6	254	9	0
	2	9.2	203	0	0
	3	10.2	241	0	8
	4	8.7	202	0	21
	5	7.4	174	0	38
10–3	1	7.4	164	9	0
	2	8.7	174	2	0
	3	8.4	177	0	0
	4	7.7	191	0	9
	5	7.0	170	0	40
10–7	1	9.7	241	26	0
	2	7.6	188	6	0
	3	8.0	200	4	16
	4	6.3	153	0	47
	5	5.0	121	0	66
5–8	1	9.7	264	20	0
	2	10.1	311	7	0
	3	9.9	251	0	0
	4	10.3	282	0	11
	5	6.4	201	0	80

WAA on average 66 and 80% of the spikelets, respectively, had shattered seeds (Table 4).

Instead of expressing the number of seeds as a percentage of the number of florets present at that moment, we were also interested to know which percentage of the initial florets resulted in a seed. Therefore, the initial number of florets was calculated from spikes that had not shattered seeds yet and valued 234, 177, 211 and 274 in genotypes 1–5, 10–3, 10–7 and 5–8, respectively. The actual number of seeds was expressed as a percentage of the initial number of florets per spike (Table 5). At one and two WAA, this percentage was generally lower than at three WAA because distal florets had not flowered yet. At four to five WAA, this percentage declined due to shattering. Consequently, three WAA is the best stage to determine biological FSU, which is defined as the percentage of initial florets producing a seed (Elgersma, 1985). Estimated values of biological FSU in genotypes 1–5, 10–3, 10–7 and 5–8 thus were 8, 72, 73 and 63%, respectively. In genotype 5–8, however, this estimation may be somewhat low because the four spikes sampled at 3 WAA contained rather few florets per spike (Table 4) and both the number of seeds per spike and the actual percentage of productive florets were low (Table 5), compared to the other sampled spikes of this genotype.

At five WAA, in genotype 1–5 only 50% (9/18) of the florets with seed at three WAA yielded seed (Table 5). In genotypes 10–3, 10–7 and 5–8, only 87, 62 and 76% of the seeds present at three WAA were still present at five WAA (Table 5). This illustrates the loss of seed caused by shattering.

Table 5. The actual number of seeds per spike, and the percentage of productive florets, calculated as percentage of actual and initial florets (A and I, respectively) in spikes of four genotypes of perennial ryegrass at five stages

Genotype	Stage (WAA)	Number of seeds per spike	Percentage productive florets (%)	
			A	I
1–5	1	19	10	8
	2	10	5	4
	3	18	8	8
	4	15	7	6
	5	9	5	4
10–3	1	98	77	55
	2	114	67	64
	3	127	72	72
	4	121	65	68
	5	110	66	62
10–7	1	129	87	61
	2	132	79	63
	3	154	80	73
	4	124	83	59
	5	95	78	45
5–8	1	153	78	56
	2	223	81	81
	3	172	69	63
	4	196	72	72
	5	131	81	48

The percentage of productive florets related to floret position

The percentage of florets containing a normally developing seed was related to floret position within the spike (Table 6). Each value was calculated from at least 25 florets. Genotype 1–5 had an extremely low percentage of productive florets. In the high yielding genotype 5–8 (Table 1), many florets contained a seed.

In spikes sampled one WAA of genotypes 10–3, 10–7 and 5–8 the percentage of productive florets was not lower in distal florets, i.e., no decline in successful fertilization was observed. At two WAA, the percentage of productive florets declined in genotype 10–3 but not in the other genotypes, i.e., embryos in distal florets were not more susceptible to abortion than in basal or intermediate florets until two WAA. At later stages, the percentage of productive florets tended to decline in distal florets in genotypes 1–5, 10–3 and 10–7, but not in genotype 5–8. On average, 5% of the unproductive florets showed seed abortion occurring later than one week after flowering in all genotypes. The percentage of aborting seeds did not increase in time and such seeds were found at all floret positions.

Discussion

Normal development

Our study gives a cytological description of normal

development of embryo and endosperm in perennial ryegrass. An exact time table cannot be presented because it is not certain on which specific day an examined floret had been pollinated.

Our results concerning normal seed development demonstrate the lag in time between early embryonic and endospermatic cell division, and agree with the observations of Reusch (1959). Division of the endosperm nucleus generally starts before the first division of the zygotic cell (Evenari, 1984). Moreover, the early growth rate of the embryo is much slower than that of the endosperm in perennial ryegrass (Reusch, 1959).

The pattern of cell divisions in the pro-embryo of perennial ryegrass has not been described before, as far as we know, and is similar to the pattern observed in barley and rye (Suetsugu, 1951). The rate of seed development depends on the position of the floret within the ear. Within each spikelet, top florets are the last to flower but first to ripen. Not surprisingly, seeds from basal florets are larger than seeds from apical florets within a spikelet (Anslow, 1964).

Table 6. The percentage of florets with a normally developing seed related to floret position in four genotypes of perennial ryegrass at five stages

Genotype	Stage (WAA)	Floret position				
		1–3	4–6	7–9	10–12	13–15
1–5	1	12	4	6	–	–
	2	8	0	7	6	–
	3	11	7	8	3	–
	4	8	5	10	3	–
	5	9	3	1	0	–
10–3	1	74	81	72	–	–
	2	75	73	61	42	–
	3	84	71	54	74	–
	4	77	66	53	21	–
	5	71	73	53	–	–
10–7	1	78	92	98	–	–
	2	80	76	85	–	–
	3	81	81	83	58	–
	4	86	88	69	–	–
	5	79	82	59	–	–
5–8	1	83	73	77	89	–
	2	83	80	75	90	–
	3	76	69	66	57	–
	4	78	70	75	59	70
	5	86	80	70	79	–

Ovule degeneration and seed abortion

Genetic variation occurred for biological FSU: in the low yielding genotype it was only 8% but in the three medium to high yielding genotypes, biological FSU ranged from 63 to 73%. It was striking, however, that although the percentage of unproductive florets differed greatly among genotypes, the histology of degeneration was similar: ovule degeneration occurring shortly after anthesis was the major cause of decreased biological FSU in all genotypes. It is not clear whether this was caused by lack of fertilization or of divisions of the primary endosperm nucleus or the zygote. Also, meiotic irregularities may contribute to sterility. This may have been important in the low yielding genotype that descends from an interspecific cross. However, we did not check meiosis.

In a low percentage of the observed seeds, we found degeneration starting at later developmental stages. Seed abortion occurred at all floret positions, this was similar in all four genotypes. The early development of such aborting seeds is normal; a globular embryo and cellular endosperm are present. In some cases, abortion may have been caused by insects, which were sometimes found on top of the seeds, inside the lemma and palea. Insect damage has also been reported in cocksfoot, where florets with thrips infestation were found, larvae destroying the developing seed (Johnston, 1960).

Our results, obtained on spaced plants, contrast with the ideas of Burbidge et al. (1978), who consider abortion of seed occurring later than three weeks after anthesis one of the major reasons for low FSU in plots of perennial ryegrass. However, Meijer (1985) observed in plots of perennial ryegrass that late abortion never exceeded 3% of the total, which agrees with our observations on spaced plants.

Floret site utilization

In our material, successful fertilization did not decline within a spikelet from the basal to the distal floret, which contrasts with the observations of Anslow (1963; 1964) and Burbidge et al. (1978).

They also found that in plots, seeds of distal florets were more susceptible to abortion. This pattern suggests a competition for assimilates or mineral nutrients within the spikelet (Hebblethwaite & Hampton, 1981; Marshall, 1985). In plots, competition between spikes for light, water, assimilates and nutrients is much stronger than in spaced plants. We found distal florets within spikes of spaced plants to produce slightly less seeds than lower florets. However, unproductive florets were found at all floret positions, which was also reported by Knowles & Baenziger (1962). This suggests genetical or cytological causes rather than physiological stress.

Low FSU is reported to be the most important limiting factor for high seed yield in many grass species (reviewed by Elgersma, 1985). For perennial ryegrass very different FSU are reported: 38% (Hill, 1971), 44% and 48% (Bugge, 1981), 53.9% (Beddows, 1931), 62% (Griffiths & Lewis, 1967) and 92.9% (Jenkin, 1931). Burbidge et al. (1978) observed that about 60% of the florets set seed within 3 weeks from anthesis, but by final harvest, after 4 to 5 weeks, only 40 to 50% of these yielded seed.

In this study, biological FSU was 8, 72, 73 and 63%, respectively, in the four genotypes. Although the percentage of florets containing a normally developing seed declined only slightly in spikes sampled one to five WAA, the actual numbers of florets and seeds per spike decreased rather strongly due to seed shattering. At five WAA only 50, 87, 62 and 76% of the initial seeds were present in the four genotypes.

Our results indicate that in spaced plants of perennial ryegrass, low biological FSU decreases seed yield. However, in seed crops economical FSU is only 15 to 20%. Although in plots biological FSU is probably lower than in spaced plants due to increased competition, we suggest that in seed crops also factors other than biological FSU, i.e., uneven flowering and ripening among spikes, seed shattering, lodging, diseases, harvest and cleaning losses, limit realized seed yields.

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CHAPTER 4

Euphytica S: 51–57 (1988)

Abscission and seed shattering in perennial ryegrass (*Lolium perenne* L.)

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Summary

Yield in grass seed crops is decreased by seed shattering, which is generally caused by abscission. Abscission layer development in perennial ryegrass, *Lolium perenne* L., was investigated to determine its histogenesis in relation to the morphological stages of the inflorescence. Spikelets were fixed with weekly intervals from heading to harvest ripeness. Florets were dissected and microtome sectioned.

Abscission layers were located in the rachilla just below each floret. They were already present at the heading stage. Only slight histological changes were observed in the cells of the abscission layers during flowering and seed development. Breaking of the abscission layers occurred four to five weeks after anthesis, starting at the epidermis. No degradation of the abscission layer cells was detected, indicating that abscission took place mechanically. No histological differences in abscission mechanism were found between groups of genotypes with contrasting seed retention, suggesting that in our material differences in seed retention were probably caused by morphological factors other than abscission layer development. Selection for high seed retention is briefly discussed.

Introduction

Perennial ryegrass, *Lolium perenne* L., is a cool season, perennial grass that is propagated by seed. It is the most important forage grass in Western Europe, and it is becoming increasingly important as a turfgrass.

In contrast to cereals, seed yield characteristics of most forage grasses have received little attention from plant breeders (Griffiths, 1965; Elgersma, 1985), despite relatively low seed yields. Low yields are due to a variety of factors including unproductive florets, seed shattering prior to harvest (Anslow, 1964) and seed loss during harvesting (Stoddart, 1964; Jensen, 1976) and cleaning (Meijer, 1985). Increased seed retention does not influence forage quality and thus would be one of the most desirable characters in grass seed crops (Griffiths, 1965). The introduction of growth regulators into grass seed crops to keep the crop upright will increase susceptibility to shedding prior to harvest, because in a lodged crop, the spikes are somewhat protected against wind. Selection for seed retention in grass breeding is, therefore, strongly recommended.

Seed shattering generally occurs by abscission and in many cereals seed retention results from elimination of the abscission layer, a process usually controlled by major genes (review by McWilliam, 1980). If abscission layers are normally deve-

loped, the degree of seed shattering is influenced by various morphological characters of the inflorescence, and variation is under polygenic control (McWilliam, 1980).

Limited information is available regarding the development of the abscission layer and the mechanism of the shattering process in forage grasses. In the present study we investigated the development of the abscission layer in perennial ryegrass in relation to the morphological stage of the inflorescence. The seed is botanically a caryopsis, enclosed by lemma and palea, but will be referred to in this paper as a seed.

Materials and methods

In 1985, diploid genotypes of perennial ryegrass, *Lolium perenne* L., were grown on a sandy soil in a spaced planted nursery at Wolfheze, The Netherlands.

The inflorescence of perennial ryegrass is a spike containing 20 to 30 spikelets. Spikelets are attached to the main stem or rachis. They consist of 5 to 13 normally developed florets with one or some rudimentary top florets. Apical spikelets have fewer florets than basal and intermediate spikelets. The florets are arranged on the rachilla in the same plane as that of the spikelets upon the rachis (Figs. 1 and 2).

Assessments were made on individual spikes from the time of heading, i.e., the day when the tip of the ear was first visible. Spikes were collected at random at one-week intervals starting at the heading stage until five weeks after the beginning of anthesis, to provide a basis for relating abscission layer development to a specific morphological stage of reproductive development. Anthesis began three to four weeks after heading.

At each harvest, three tillers were fixed in FAA (900 ml 15 M ethanol, 50 ml acetic acid, 50 ml formaldehyde l^{-1}) and stored at 4° C in 15 M ethanol. The three central spikelets of each inflorescence were taken to reduce variation due to position and developmental stage. Within each spikelet, the central florets or seeds were dissected together with a part of the rachilla. The material was dehydrated in a tertiary butyl alcohol series, embedded in Epon, and sectioned at 5 μm longitudinally, perpendicular to the axis of the rachilla, with a glass knife in a LKB ultramicrotome. Some cross sections were also made. The material was stained with toluidine blue and studied with a light microscope. Lignification of cell walls was checked in several sections with phloroglucinol-HCl two weeks after heading and five weeks after anthesis.

In a preliminary investigation of abscission layer histogenesis, fourty-six florets of various genotypes were sectioned at seven developmental stages, starting at heading till six weeks later. In a second experiment, florets were sectioned in later stages to study the abscission mechanism. Seven genotypes were chosen, that had been examined for seed retention during 1982 and 1983. Seed retention of spaced plants was determined by tagging twenty similar spikes at anthesis, and harvesting ten spikes at harvest ripeness and ten spikes two weeks later. Seed retention was calculated by expressing the yield of the second sample as a percentage of the yield of the first sample. Four genotypes had a high seed retention in 1982 and 1983. Three other genotypes had a relatively low seed retention during these years (Table 1). In each group of genotypes, a number of florets was sectioned at both four and five weeks after anthesis, of which 13 florets in each group were observable.

Table 1. Seed retention of seven genotypes of *Lolium perenne* L. as determined in 1982 and 1983 on spaced plants

Genotype	Source	Seed retention (%)		Class
		1982	1983	
1	Ecotype*	100	100	high
2	'Perma'	94	93	high
3	'Sprinter'	87	100	high
4	'Lamora'	100	100	high
5	'Lamora'	69	37	low
6	'Compas'	28	63	low
7	'Lamora'	35	74	low

* Collected in Limburg, The Netherlands.

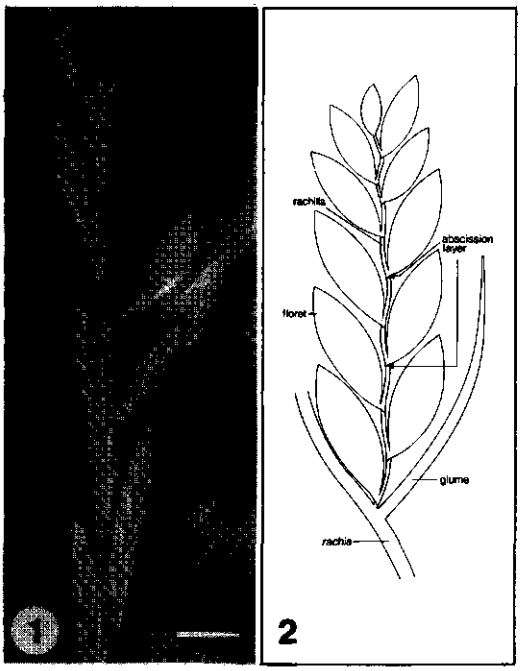

Fig. 1. Spikelets with florets. Scale bar, 2.5 cm (× 0.4).

Fig. 2. Schematic drawing of a spikelet, showing the position of the abscission layers across the rachilla, just below each floret.

Results

Abscission layer development

Abscission layers were already present at heading. They are located below each floret across the rachilla, just below the attachment of the palea and the lemma on the rachilla (Figs. 2 to 7). The cells of the abscission layer can be recognized because they are rather small compared to the parenchyma cells in the rachilla, they have a rectangular shape and a regular position (Figs. 3 and 5). From cross sections, it appeared that three vascular bundles are present in the ranchilla (Fig. 4). Therefore, we can distinguish a central area between the three vascular bundles and an outer area between the vascular bundles and the epidermis. During the heading stage, in longitudinal sections the abscission layer consists of two cell layers at the epidermal side. In the outer area, adjacent to the vascular bundles 2 to 6 cell layers could be seen, and in the central area the abscission layer varied from 4 to 8 cells wide. The cell walls in the outer area stained more intensely than walls of the cells of the abscission layer in the central area (Fig. 5).

During seed development a slight increase of the number of cell layers in the central area of the ranchilla to a maximum of 13 was observed, probably resulting from cell divisions (Fig. 5). After ligning staining, we observed that the cell walls of the abscission layer were not lignified, but in the glumes some lignin staining occurred.

Abscission mechanism

The abscission mechanism was studied in two groups of contrasting genotypes. When dissecting the seeds for the anatomical study, we found that five weeks after anthesis many seeds had already shattered. It proved difficult to handle the spikelets without causing artificial seed shattering. Therefore, only the relatively low number of 13 florets per groups resulted in useful sections for observation.

No anatomical or histological differences in vascularization and development of abscission layers were found between the two groups of genotypes.

The abscission mechanism is illustrated in Figs. 6 and 7. Separation of the seed from the ranchilla takes place between two cell layers of the abscission zone, starting at the epidermal sides (Fig. 6). The seed is connected to the rachilla with the vascular bundles and the central area. Finally, the vascular bundles break and the central area detaches from the rachilla (Fig. 7). The seed abscises and is free to shatter.

Discussion

Location of abscission layers

This investigation revealed that seed shattering in perennial ryegrass is conditioned by the formation of abscission layers that extend across the rachilla at the base of each floret. This pattern is typical of many temperate grass species (McWilliam, 1980). In many tropical grasses, the rachis disarticulates immediately below the glumes, resulting in the

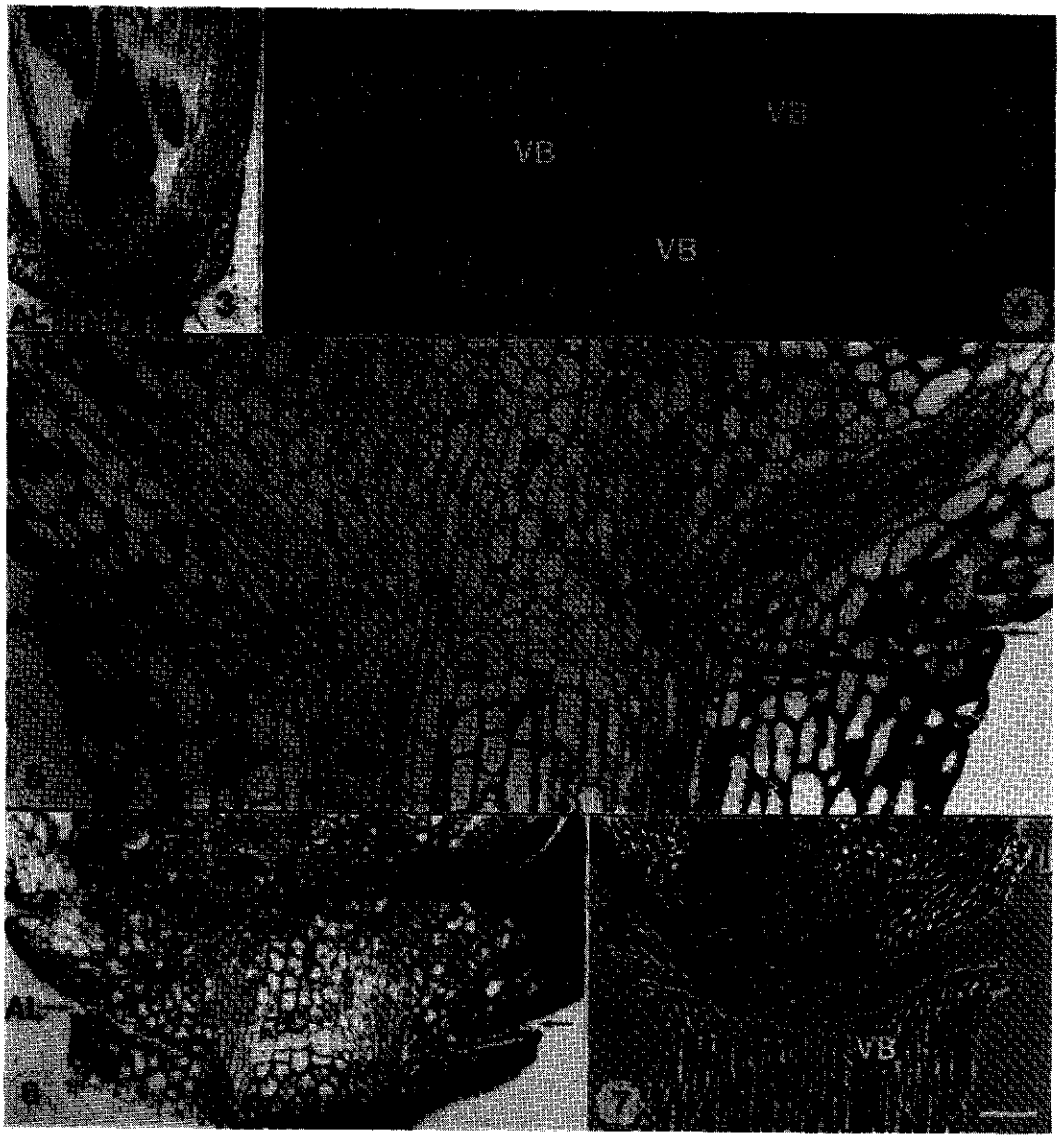

Fig. 3. Section in the pre-anthesis stage showing the anthers (A), the ovary (O) and the abscission layer (AL), indicated by arrows, across the rachilla. Scale bar, 0.5 mm (× 20).

Fig. 4. Cross section through the rachilla, showing three vascular bundles (VB). Scale bar, 72.5 μm (× 138).

Fig. 5. Section at two weeks after heading showing the abscission layer (arrows). Two vascular bundles (VB) can be seen. Scale bar, 72.5 μm (× 138).

Fig. 6. The opening of the abscission layer (AL) at five weeks after anthesis starts from the epidermis (arrows). In the center a cambium (C) is visible. Scale bar, 0.12 mm (× 83).

Fig. 7. Completely separated seed, cells in the central area of the abscission layer (AL) and the vascular bundles (VB) are torn apart, at the epidermal sides smooth cell walls are visible. Scale bar, 0.2 mm (× 50).

shedding of a whole spikelet, usually containing only one carvopsis (reviewed by McWilliam, 1980).

In *Paspalum notatum* and *Paspalum dilatatum* (Burson et al., 1978) and in *Panicum maximum* (Weiser et al., 1979) the abscission layer, located below the whole spikelet across the pedicel, is referred to as primary abscission layer. In *Panicum maximum*, below the fertile floret across the rachilla, another abscission layer is found, referred to as a secondary abscission layer (Weiser et al., 1979). Both primary and secondary abscission layers are also found in *Panicum coloratum* (Burson et al., 1983) and in *Phleum pratense* (Bean, 1965).

Sequential development

At heading, abscission layers were already present in perennial ryegrass, as in *Alopecurus pratensis* (Woehrmann, 1958). Also, secondary abscission layers in *Panicum maximum* and *P. coloratum* (Burson et al., 1983) were first visible at heading, but the primary abscission layers in *Paspalum* (Burson et al., 1978) and *Panicum* (Weiser et al., 1979; Burson et al., 1983) were already initiated during the boot stage.

In perennial ryegrass, at the epidermal side, two cell layers were present, but in the center of the rachilla, adjacent to and between the three vascular bundles, we found more layers. During flowering and ripening, the number of cell layers in the central area between the vascular bundles increased slightly. In *Paspalum* and *Panicum*, primary abscission layers were wedge shaped, i.e., five to seven cell layers wide near the epidermis, and gradually narrowing to one to three cells adjacent to the vascular tissue near the center of the pedicel (Burson et al., 1978; 1983; Weiser et al., 1979). This pattern is opposite in terms of number of cell layers to our observations in perennial ryegrass.

We noticed that cell walls in the layer between the epidermis and the vascular tissue stained intensely, but cell wall thickening during development was not found. In *Paspalum* and *Panicum*, thickening of cell walls in the primary abscission layer was observed during reproductive development.

In perennial ryegrass, lignification of cell walls in the abscission layer did not occur. Woehrmann (1958) mentioned thickening of membranes and sclerification of cells in both the pedicel and in the lower cell layers of the abscission layer in *Alopecurus pratensis*. The abscission layer broke between lignified and non-lignified cells. In a non-shattering mutant obtained by irradiation, the abscission layer was present, but lignification did not occur (Woehrmann, 1958). In *Panicum maximum*, the secondary abscission layer consisted of two to four layers of very small, highly lignified cells (Weiser et al., 1979). Sclerification of the secondary abscission layer in Panicum started from the epidermis and continued inwardly (Burson et al., 1983).

The abscission mechanism

Because there is variation in flowering time within inflorescences, among inflorescences of an individual plant, and among plants in a population, maturation of a seed crop occurs over a period. In seed crops, initial seed shattering is generally observed 3.5 to 4 weeks after the beginning of anthesis depending upon weather conditions, i.e., wind and rainfall. Shattering begins with upper seeds of apical spikelets and continues with upper seeds of intermediate spikelets and central seeds of apical spikelets, and so on. In our material and under our conditions, initial seed shattering started 3.5 to 4 weeks after the beginning of anthesis in upper spikelets. In perennial ryegrass c.v. 'S.24' shattering had already begun 2.5 weeks after anthesis and seeds were lost at a constant rate (Anslow, 1964). The discrepancy between the two results could have been due to differences in methodology. We observed individually labelled spikes but Anslow did not. Considerable variation between spikes in flowering time is known to occur in perennial ryegrass seed crops. Lower humidity prevailing during Anslow's experiment also may have contributed to the earlier shedding.

Two general mechanisms of seed abscission have been reported in the literature (Pfeiffer, 1928): 1. disintegration of the middle lamella and cell walls as a result of (bio)chemical changes, and 2. mechanical tearing enabled by weak tissue in the layer.

In *Paspalum* spp. and *Panicum* spp., the primary abscission layer disintegrates biochemically accor-

ding to Burson et al. (1983) and Weiser et al. (1979). Cells elongated and collapsed and lacunae were formed, starting from the center of the pedicel toward the periphery. Later the vascular bundles were crushed and finally the epidermal cell walls disintegrated, causing abscission of the spikelet (Burson et al., 1978).

In our material no evidence of cell wall degradation was observed, similar to secondary abscission layers in *Panicum* (Burson et al., 1983), indicating that separation results from middle lamellar separation or mechanical force. Fahn and Werker (1972) state, that no clear distinction can be made between biochemical and mechanical abscission types and there are actually all kinds of transitory stages. According to Osborne (1984), grass seeds separate actively, cell separation being induced by abscisic acid. This discrepancy can only be resolved with more precise techniques.

Plant breeding perspectives

In cereals many non-shattering mutants with a partly developed abscission layer or with no abscission layer at all have been identified (McWilliam, 1980). Clearly, the seed retaining genotypes observed in this study are not such non-shattering mutants, because an abscission layer is present, and the abscission mechanism cannot be distinguished from that of the seed shattering genotypes. Probably, variation for seed retention as observed in perennial ryegrass (this study) and in Italian ryegrass (Harun and Bean, 1979) is not related to the abscission layers but to various indirect factors influencing shattering, i.e., the size, strength, shape, and flexibility of the glumes and compactness of the inflorescence and/or spikelets (McWilliam, 1980). However, in this preliminary experiment we did not measured such characters.

The seed retention measurement were made in 1982 and 1983, but spikelets were collected for sectioning in 1984, during a wet summer. It is possible that the differences between plants which occurred in 1982 and 1983 were not fully expressed in 1984. The expression of seed retention may have been modified by environmental conditions. In perennial ryegrass, seeds shatter only when maturation, senescence and dehydration are well advanced. In practice, empty florets are difficult to separate during threshing because they stick to the underlying floret, indicating that the abscission layer breaks only when a filled seed is present. In a large spaced-planted population of perennial ryegrass, seed yield and seed shattering were strongly correlated ($r = 0.70^{**}$, Elgersma, unpublished results). High yielding plants are likely more susceptible to shattering, if harvest is delayed. When many seeds are set in a spikelet, the total weight pressing the underlying abscission layers increases, which may result in easier breaking of the lower abscission layers. Consequently selection for seed retention must be carried out simultaneously with selection for yield.

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CHAPTER 5

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Genetic Variation for Seed Yield in Perennial Ryegrass (*Lolium perenne* L.)

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With 4 figures and 5 tables

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Abstract

In perennial ryegrass seed yield is low and unreliable and little is known about the seed-yielding capacity of different cultivars. Therefore, genetic variation for seed yield was studied for three years at two locations. Twelve trials consisting of drilled plots of nine diploid, late-flowering cultivars in four replications were harvested for seed. Significant genetic differences for seed yield and seed weight were detected. The ranking of the cultivars for seed yield differed from that for seed weight. The levels of seed yield and seed weight were affected by such environmental factors as year, soil type and crop production year, but interactions of these factors with cultivars were generally not significant. Variation in seed yield was more related to variation in seed number than to variation in seed weight. There were no significant cultivar differences for yield stability. The highest yielding cultivar was superior over a wide range of environments and the seed yield of the poorest cultivar was on average only 64 % of that of the best cultivar.

Key words: *Lolium perenne* — perennial ryegrass — seed yield — genetic variation — seed weight — stability analysis

Perennial ryegrass, *Lolium perenne* L., is the most important cultivated grass species in NW-Europe. Many cultivars have been bred with excellent forage or turf qualities. Seed-yielding capacity has received little attention from plant breeders (GRIFFITHS 1965, VYNCKE 1981), despite its relatively low level (HEBBLETHWAITE et al. 1980, BUGGE 1987). This may be due to the opinion that seed and forage production are negatively correlated (VAN WIJK 1980). Recently, improved seed yielding capacity has become a more important selection criterion in grass breeding, since it is of considerable importance in the cost of seed multiplication and ultimately determines the commercial success of a cultivar, regardless of its forage or turf qualities (ELGERSMA 1985). In addition, high performance for seed yield should be reliable over a wide range of environmental conditions. Significant cultivar differences in seed yield of perennial ryegrass were found in the United Kingdom (EVANS and MUNCEY 1977) and in Denmark (NORDESTGAARD and JUEL 1979). Only limited and confidential information is available on the seed yield of cultivars obtained in practice in the Netherlands, and these data are rather unreliable due to differences in region, seed crop management, acreage, soil type, etc. (Anonymous, 1985).

Selection for seed yield is a major problem (VAN WIJK and DUYVENDAK 1984). Therefore, an extensive study was carried out to define selection criteria for the genetic improvement of seed yield in perennial ryegrass. Results are reported here on (i) genetic differences in seed yield, (ii) relations between seed yield, seed weight and seed number and (iii) the effects of year, location and crop production year on stability of seed yield.

Materials and Methods

Plant material and experimental design: The trials were carried out from 1985 to 1988 in the experimental fields 'Born' and 'Nude' at Wageningen, the Netherlands, on sandy and clay soil, respectively. Nine diploid perennial ryegrass cultivars of the pasture type were used (Table 1). The cultivars were chosen because of similar ear emergence dates, comparable vegetative production of a high level, contrasting seed production and different origin/genetic background.

Certified basic seed was kindly provided by the various breeding companies and stored at 4 °C. The same seed lots were used in all experiments. Germination tests revealed that the germination capacity of the seed remained at a high level during these years.

The seed was sown in the autumns of 1985, 1986 and 1987 at 10 kg ha^{-1} with a row width of 0.25 m in 6-row plots of 1.5 m × 10.5 m. In 1985, at each location three trials (A, B and C) were established: on sandy soil SA, SB and SC and on clay soil CA, CB and CC. Each trial had a complete randomized block design with four replications and was surrounded by border plots, sown with cv. 'Wendy'. Thus each trial was 57 m × 10.5 m. This design allowed application of fertilizer and herbicide to the crop without causing mechanical damage. Plots could be harvested individually and border effects were minimized. New trials were established in 1986 and 1987 (Table 2).

Experimental management: The seed was sown in open land. In the Netherlands, about 50 % of the perennial ryegrass seed crops are sown under a cover crop, e.g., winter wheat, peas or flax. Fifty percent are sown in open land in autumn, as was done in this experiment to avoid unnecessary complications. Details of the experimental management are given in Table 2. Throughout the years, the previous crop on sandy soil was spring barley, while on clay soil field beans had been grown for green manure prior to our experiments. Agronomic seed crop management was according to recent recommendations of the Research Station for Arable Farming and Field Production of Vegetables (PAGV) (MEIJER and VREEKE 1988), in order to simulate large-scale, commercial production.

After the seed harvest of the first year crops in 1986 and 1987, the stubbles were cut and the trials were used the next year for experiments with second year crops. In the Netherlands, about 50 % of the commercial perennial ryegrass seed production fields are also harvested the second year.

Seed yield: In 1986 the two outer rows of each plot in trials SA and CA were cut by hand approximately one week before harvest and only the four central rows were harvested (1 m × 10.5 m). In all other trials, the complete plots (1.5 m × 10.5 m) were harvested. The lodged inflorescences between plots were carefully put aside by hand about one week before harvest, to separate the plots and allow machine harvesting. In contrast to the situation in practice, where crops are swathed or sometimes directly combine-harvested, the crop was cut with a Hege fodder harvester (reciprocating knife mower) to minimize harvest losses. The crop of individual plots was cut at harvest ripeness, at 43 to 50 % moisture, put in large jute bags and dried at 25 °C to about 11 % moisture. The seed was threshed and cleaned to at least 98 % purity. Yields are converted to kg ha^{-1} at 11 % moisture. Thousand-grain weight (tgw) was assessed by weighing three samples

Table 1. Abbreviations used in this paper and names of nine *Lolium perenne* cultivars, year of registration in a cultivar list, heading date, breeder, relative dry matter yield (DMY) and relative seed yield (SY)

Cultivar Abbrev.	Cultivar Name	Registr. year	Breeder	Heading date	DMY (%)*	SY (%)**
S	'Semperweide'	1946	Zwaan en de Wiljes (NL)	7-6	96	92
W	'Wendy'	1979	Van der Have (NL)	8-6	100	105
C	'Compas'	1958	Van Engelen & Joordens (NL)	8-6	98	105
L	'Lamora'	1945	Mommersteeg (NL)	9-6	99	97
Pe	'Perma'	1939	Cebeco — Handelsraad (NL)	9-6	101	93
B	'Barenza'	1949	Barenbrug (NL)	9-6	97	96
Pa	'Parcour'	1969	Petersen Saatzucht (BRD)	9-6	100	—
T	'Trani'	1977	Dansk Planteforaedling (DK)	10-6	—	—
V	'Vigor'	1948	Rijksstation Voor Plantenveredeling (B)	12-6	101	113

* Averaged over all diploid late-maturing cultivars in the Dutch Descriptive List of Varieties (ANONYMOUS, 1984).

** Averaged over the seven cultivars mentioned here from 1971 to 1983 (source: ANONYMOUS, 1985).

Table 2. Experimental management

Location Trials	Sand SA, SB, SC	Clay CA, CB, CC	Sand SD	Sand SE	Clay CE, CF
First-year crop					
Sowing date	2 Sep. 1985	26 Sep. 1985	5 Sep. 1986	16 Sep. 1987	16 Sep. 1987
Fertilizer					
date	August 1985	—	August 1986	August 1987	—
rate (kg/ha)	30 N		30 N	30 N	—
date	4 March 1986	4 March 1986	18 Feb. 1987	8 March 1988	4 March 1988
rate (kg/ha)	80 N	80 N	150 N	130 N	150 N
	150 P, 150 K	150 P, 150 K	125 P, 225 K	60 P, 108 K	60 P, 108 K
date	17 March 1986	17 March 1986	—	—	—
rate (kg/ha)	70 N	50 N	—	—	—
Seed harvest					
trials	SA, SC	CA, CC	SD	SE	CE
date	29 July 1986	31 July 1986	4—7 Aug. 1987	19—25 July 1988	25—28 July 1988
Second-year crop					
Weed control	Tribunil	Tribunil	Tribunil		
date	Sep. 1986	Sep. 1986	Oct. 1987		
rate (l/ha)	3	3	3		
Fertilizer					
date	1 Oct. 1986	1 Oct. 1986	1 Oct. 1987		
rate (kg/ha)	45 N	45 N	45 N		
	38 P, 68 K	38 P, 68 K	38 P, 68 K		
date	18 Feb. 1987	18 Feb. 1987	4 March 1988		
rate (kg/ha)	150 N	130 N	150 N		
	125 P, 225 K	125 P, 225 K	60 P, 108 K		
Seed harvest					
trials	SB, SC	CB, CC	SD		
date	4—7 Aug. 1987	7—11 Aug. 1987	19—25 July 1988		

of 100 seeds. These samples were also used in germination tests on a 'Copenhagen' table, set at 30/20 °C and a photoperiod of 16/8 h. Seed number was calculated from seed yield and thousand-grain weight.

Other observations: Ear emergence date, various stages of ear elongation and time of anthesis were assessed three times a week. Plots were considered to be at peak anthesis when the majority of the ears had anthers exerted throughout the spike. Pollen samples were collected during 1986 and 1987 and stained with lactophenolic acid fuchsine. Lodging was visually rated three times a week.

Statistical analyses: In each trial analyses of variance were conducted for the characters observed. Also, various trials were combined and analyses of variance were carried out to detect cultivar — location (CL), cultivar — year (CY), cultivar — crop production year (CP), cultivar — harvest method (CH) and three-way interactions. Simple correlations were calculated between characters on a plot basis. A stability analysis was conducted for seed yield. In the analysis of variance, the average error was estimated from the individual trials. The observed phenotypic mean values of cultivar i in environment j were regressed on the means of the environments to obtain a coefficient of regression, b_i, for each cultivar (FINLAY and WILKINSON 1963). In addition the deviation mean squares (s^2_{di}) that describe the contribution of cultivar i to cultivar-environment interactions were calculated (EBERHART and RUSSELL 1966).

Results

Genetic variation

Crop development: Although cultivars with similar ear emergence dates had been chosen, significant differences occurred for time of ear emergence, ear elongation and flowering (data not given). 'Perma' was earlier and 'Lamora' and 'Barenza' were later than expected from Table 1: in all trials 'Perma' was the earliest

Table 3. Seed yield (kg ha^{-1}) of nine perennial ryegrass cultivars in 12 trials at two locations during three years. The cultivars are ranked for mean seed yield

Cultivar	Trial											
	1986				1987					1988		
	SA-1*	SC-1	CA-1	CC-1	SD-1	SB-2	SC-2	CB-2	CC-2	SD-2	SE-1	CE-1
'Trani'	1284	1580	1868	2293	1425	917	1111	1404	1693	666	829	1066
'Wendy'	1194	1431	1865	2242	1410	937	1079	1342	1722	455	782	904
'Semperweide'	1030	1231	1844	2059	1476	951	956	1250	1538	459	825	860
'Vigor'	1199	1390	1897	2138	1340	847	855	1217	1270	575	636	910
'Parcour'	965	1221	1609	1899	1258	908	1066	1533	1599	561	781	797
'Perma'	829	1317	1691	2084	1284	805	1027	1353	1677	403	771	901
'Compas'	992	1406	1838	2096	1217	813	804	1137	1556	502	595	635
'Barenza'	1102	1210	1604	2113	1011	567	587	1060	1147	383	498	756
'Lamora'	1073	1151	1496	1558	927	539	555	921	1186	387	377	581
Mean	1074	1326	1746	2055	1261	809	893	1246	1488	488	677	823
LSD (5 %)	218	159	187	222	183	130	193	209	185	228	291	363
cv (%)	14	8	7	7	10	11	15	12	9	32	29	30

* Trial and crop production year (−1, −2) are indicated.

cultivar followed by 'Semperweide', 'Wendy' and 'Parcour'. 'Vigor' was rather late, and 'Barenza' and 'Lamora' were the latest. The difference between 'Perma' and 'Barenza' varied from three to ten days in the various trials. In 1986 and 1987, the average pollen stainability was over 90 % and no significant differences occurred among cultivars. Lodging started at the beginning of anthesis and was complete at the end of anthesis. The lodging behaviour of the cultivars was the same, i.e., the ranking orders of the cultivars for flowering date and lodging were similar.

Seed yield: Significant differences for seed yield occurred among cultivars in each of the 12 trials (Table 3). The coefficients of variation were small in 1986 and 1987; the high values in 1988 may have been caused by an irregular stand of the crop, possibly due to fertilizer application in a rather late physiological stage compared with 1986 and 1987 (Table 2).

Seed weight and seed number: Analyses of variance within trials showed significant differences among cultivars for thousand-grain weight in each trial except CE-1. The ranking order of the cultivars across trials was similar. 'Semperweide' and 'Wendy' always had the highest seed weight and 'Compas' and 'Barenza' the lowest.

Significant differences occurred for the calculated number of seeds in 1986 and in 1987, but not in 1988. The ranking order of the cultivars for seed number was more variable among trials than that for seed yield or seed weight, but 'Trani' generally had the highest seed number and 'Lamora' the lowest. Within trials, no significant differences occurred among cultivars for the germination percentage of the harvested seed.

Correlations: Correlations were calculated per year for sandy and clay soil on a plot basis and revealed that ear emergence, ear elongation, flowering and ripening were all positively correlated. Thousand-grain weight was positively correlated with earliness. In 1986, seed yield was not significantly correlated with other characters, but in 1987 and 1988 yield was positively correlated with earliness. It should be emphasized, however, that only limited variation for earliness is present in this material, since it was selected for similar ear emergence dates.

The correlations between thousand-grain weight and seed yield varied among environments and were generally low: in 1986 they were not significant, in 1987 they were positive ($p \leq 0.01$) on sandy and clay soil ($r = 0.42^{**}$ and 0.37^{**}, respectively) and in 1988 the correlation was negative ($r = -0.31^{**}$) on sand and not significant on clay.

The correlations between seed number and thousand-grain weight varied from non-signif-

icant to negative, whereas the correlations between seed number and seed yield were extremely high, but it should be kept in mind that seed number was calculated from seed yield and thousand-grain weight.

The relation between seed yield and thousand-grain weight of the cultivars averaged over all environments is shown in Fig. 1.

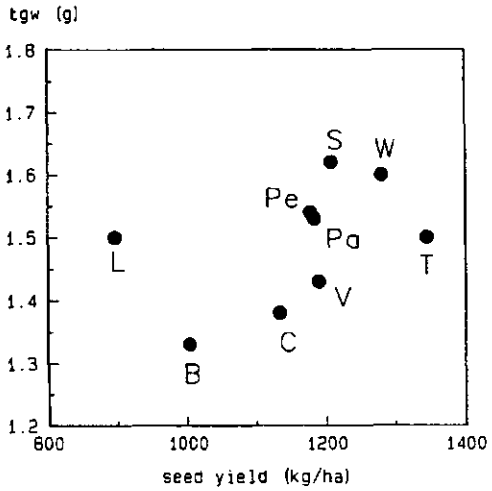

Fig. 1. The relation between seed yield and thousand-grain weight (tgw) of nine perennial ryegrass cultivars, averaged over twelve environments. For abbreviations see Table 1

Environmental effects

The environmental factors: harvest method (H), year (Y), location (L) and crop production year (P) affected the level of seed yield, seed weight and seed number. Values were highest in 1986 and lowest in 1988 and higher on clay than on sandy soil. Crop development was almost similar within years between trials, but differed among years (Table 2). Seed yields were significantly lower when border rows were cut. The cutting method was very labour-intensive, a smaller area was harvested and ripening was irregular since the two outer rows dried faster than the two central rows. Thus whereas the initial idea was to eliminate possible border effects, inaccuracy increased and therefore the cutting method was abandoned.

Second-year crops yielded about 20 % less than first-year crops. In 1987, second-year crops showed much winter damage, whereas the first-year crop was hardly affected by the severe winter. In 1988, no winter damage occurred, but the second-year crop has a very irregular stand.

The thousand-grain weight of seeds was almost equal in 1986 and 1987, but much lower in 1988. Seeds from clay had a higher thousand-grain weight and germinated slightly better (98 %) than seeds obtained from sandy soil (95 %).

The relation between average yield and average thousand-grain weight is shown in Fig. 2.

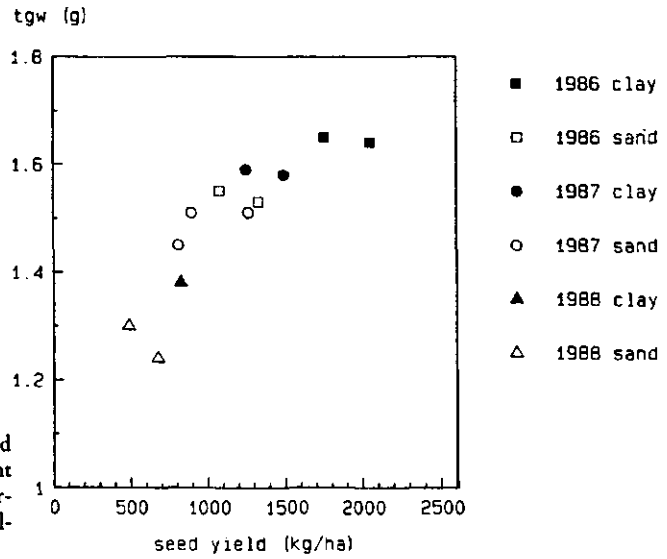

Fig. 2. The relation between seed yield and thousand-grain weight (tgw) in twelve environments, averaged over nine perennial ryegrass cultivars

Cultivars showed a tendency for increased thousand-grain weight with improved environments. The effects of year and soil type on yield and thousand-grain weight are clearly visible.

Yield stability

The ranking order of the cultivars for seed yield was more or less similar in the various trials (Table 3). The seed yield of the poorest cultivar, 'Lamora', was on average only 64 % of that of the best cultivar, 'Trani'. A stability analysis was conducted by regression of the seed yield per cultivar per trial on the mean of all cultivars per trial. The analysis of variance is shown in Table 4. Although significant, the cultivar — environment interaction was relatively small.

Table 4. Analysis of variance for seed yield of nine perennial ryegrass cultivars in twelve environments

Source	Df	SS	MS
Cultivars (C)	8	1769903	221238**
Environments (E)	11	20643894	1876718**
C × E	88	1055090	11990**
Regressions	8	142941	17868*
Deviations	80	912149	11402**
Total	107		
Average pooled error	(264)		5781

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

To specify the environmental factors causing the cultivar — environment interaction, analyses of variance using subsets of trials were conducted on a plot basis. The cultivar — harvest method interaction was significant ($p \leq 0.05$). The cultivar — year interaction was not significant in first-year crops, but it was significant in second-year crops ($p \leq 0.01$). This was mainly caused by the winter-weak cultivar 'Vigor', that performed relatively poorly in 1987 after a severe winter, compared to 1988 following a mild winter. The cultivar — location interaction was significant in 1986 ($p \leq 0.05$), but not in 1987 and 1988. Analyses of variance across years indicated that the cultivar — location, cultivar — crop production year and three-way interactions were not significant.

Fourteen percent of the interaction sum of squares were explained by the regression model (Table 4). The estimates of the stability parameters b and s^2_d are presented for each cultivar in Table 5. The percentage of variance accounted for varied from 91 to 99 %. There were no significant differences in b and s^2_d among the cultivars. As an example, the response of three cultivars with the most extreme reactions to different environments is illustrated in Fig. 3. No clear relation was found between the mean seed yield and the regression coefficient of the nine cultivars (Fig. 4).

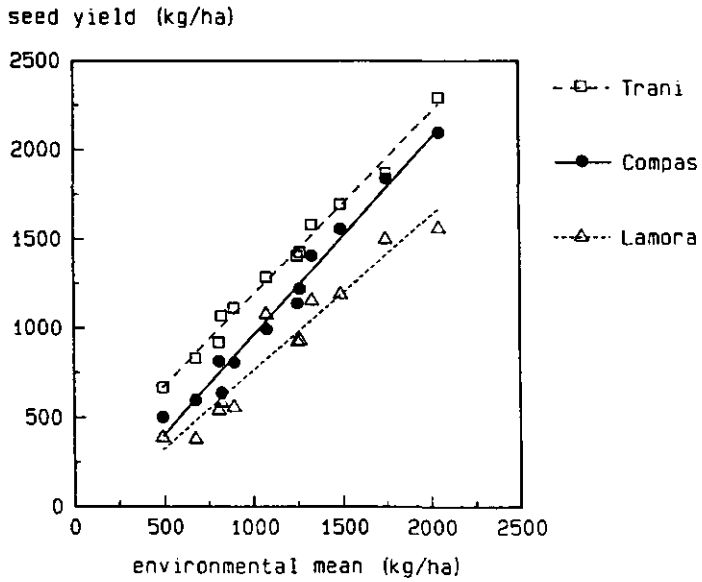

Fig. 3. The seed yield of three perennial ryegrass cultivars in twelve environments and the fitted regression lines

Table 5. The mean seed yield (kg ha^{-1}), the regression coefficient $b_i \pm SE$ and the deviation mean squares s^2_{di} of nine perennial ryegrass cultivars in twelve environments

Cultivar	Mean	$b_i \pm SE$	s^2_{di}
'Trani'	1345	1.02 ± .03	2503
'Wendy'	1280	1.09 ± .04	3133
'Semperweide'	1207	0.98 ± .06	8708
'Vigor'	1189	1.01 ± .07	11156
'Parcour'	1183	0.86 ± .08	15889
'Perma'	1178	1.02 ± .08	14559
'Compas'	1133	1.11 ± .05	6031
'Barenza'	1003	1.06 ± .08	16113
'Lamora'	896	0.87 ± .08	13187

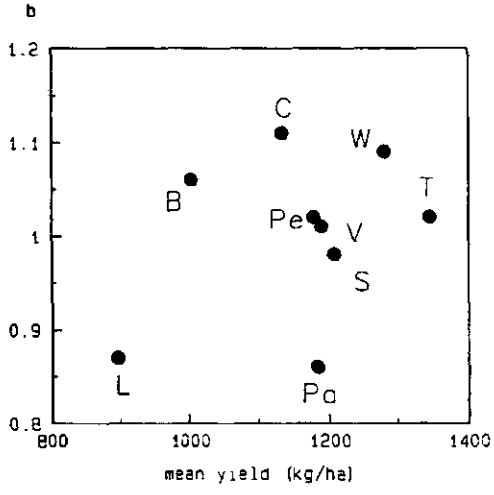

Fig. 4. The relation between the overall cultivar mean and the regression coefficient b for nine perennial ryegrass cultivars in twelve environments. For abbreviations see Table 1

Discussion

The results of these experiments establish that genetic variation for seed yield is present in perennial ryegrass and that the highest yielding cultivar was superior over a wide range of environments.

The most urgent constraints in grass breeding have been named as those connected with the assessment of herbage and seed-yielding capacity and with the selection for seed yield (VAN WIJK and DUYVENDAK 1984). Because the ranking of cultivars was fairly stable across trials, our way of assessment of seed yield appears to ensure reliable data on the relative seed-yielding capacity of perennial ryegrass cultivars. The ranking order of the cultivars is not identical to the ranking found in commerce averaged over the period 1971—1983. However, the data from commerce are incomplete as the best cultivar is missing and less reliable due to many environmental factors. Seed-crop management may not be the same in all seed companies and differs among seed growers, while in this experiment all cultivars received the same agronomic treatment. Our plot harvest method reduced losses due to shattering and combine harvesting that normally occur in commercial seed production. A different ranking order in practice may indicate that such losses are important. Another reason for the discrepancy might be associated with the pollen source. Perennial ryegrass is a self-incompatible, wind-pollinated species. In this experiment, cultivars were partly pollinated with pollen from other cultivars in neighbouring plots, whereas in commercial seed production only own pollen is available. However, as the cultivars are based on a sufficient number of cross-compatible clones, lack of compatible pollen in commercial seed production is not likely. Therefore, we do not believe pollen source differences are responsible for the observed discrepancies.

The relationship between yield and seed weight is of interest, because final yield is determined by seed number as well as by seed weight. The ranking order for seed yield differed from that for seed weight. For example, 'Vigor' and 'Parcour' had higher yields than 'Lamora', but 'Vigor' had the lowest seed weight. Consequently, 'Vigor' had a high and 'Lamora' a low seed number. Similar results were found by EVANS and MUNCEY (1977) and NORDESTGAARD and JUEL (1979). Apparently, in some cultivars the number of seeds contributes more to yield and in others the seed weight is a more important yield factor.

Both seed yield and seed weight are genetically determined and also influenced by the environment. Accross trials, seed yield was associated with seed weight due to environmental effects. For example, on clay soil both seed yield and thousand-grain weight were higher than on sandy soil, which may be related to factors such as soil structure, soil

moisture content or available minerals. Soil type affected seed yield more than seed weight, for instance in 1986 and 1987 seed yield on sandy soil was only 63 % of that on clay soil but seed weight on sandy soil was 93 % of that on clay soil. Variation in seed yield was more related to variation in seed number than to variation in seed weight.

On sandy soil, second-year crops yielded about 30 % less than first-year crops, but the thousand-grain weight was similar. Consequently, second-year crops had a lower seed number than first-year crops.

Contrary to the findings of EBERHART and RUSSELL (1966) in maize and FINLAY and WILKINSON (1963) in barley, in our trials with perennial ryegrass no clear relation was found between the average yield and the regression coefficient of the nine cultivars, indicating that high yield potential and yield stability were not mutually exclusive within the range of environments used. Yield stability may result from various mechanisms, e.g., genetic heterogeneity, yield component compensation, stress tolerance or the capacity to recover rapidly from stress. All cultivars used in this experiment are synthetics, i.e., heterogeneous populations of heterozygous genotypes. Such populations are generally well buffered against environmental influences (BECKER and LEON 1988). Our observations on spaced plants (not reported here) reveal that extensive genetic variation is present within each cultivar and there are no differences in genetic heterogeneity of the nine cultivars. Most breeders kindly provided confidential information about the origin and the number of clones on which their cultivar was based. There was no relation between the number of clones and seed yield. Some cultivars are related, but differed in seed yield. The effect of environmental stress on seed yield varies depending upon the growth stage in which occurs. Therefore, cultivars with minimal differences in maturity were chosen. Most cultivar by environment interactions were not significant, indicating that cultivars were generally affected to the same extent by environmental factors.

The prevailing opinion among forage grass breeders is that late-flowering cultivars producing high dry-matter yields often have a poor reproductive efficiency, as reported by BUGGE (1987). This study reveals that significant differences for seed yield exist among nine late-flowering perennial ryegrass cultivars with high dry-matter production and satisfactory agronomic performance, indicating that high seed-yielding capacity and high dry-matter yield are not exclusive. Plant breeders should therefore be able to combine excellent vegetative qualities and a high and stable seed production level within their new cultivars. Implications for selection on a single plant basis will be discussed in a forthcoming paper.

Zusammenfassung

Genetische Variation des Samenertrages von Deutschem Weidelgras (*Lolium perenne* L.)

Der Samenertrag von Deutschem Weidelgras ist niedrig und unzuverlässig. Überdies ist nur wenig über die Ertragsfähigkeit der verschiedenen Sorten bekannt. Deshalb wurde die genetische Variation des Samenertrages im Verlauf von 3 Jahren an zwei Orten untersucht. In 12 Drillsaatversuchen wurden von 9 diploiden, spätblühenden Sorten in 4 Wiederholungen Samen geerntet. Die Samenerträge und das Samengewicht lagen auf Lehmböden höher als auf Sandböden. Die Erträge waren 1986 am höchsten und 1988 am niedrigsten. Die Erträge zweijähriger Pflanzen lagen auf sandigen Böden ungefähr um 30 % niedriger als diejenigen von einjährigen. Bei den Merkmalen Samenertrag und Samengewicht traten signifikante genetische Unterschiede auf. Die ertragsreichste Sorte erwies sich in verschiedenen Umwelten als überlegen.

Der Samenertrag der schlechtesten Sorte brachte im Durchschnitt nur 64 % des Ertrages der besten Sorte.

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CHAPTER 6

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Seed yield related to crop development and to yield components in nine cultivars of perennial ryegrass (*Lolium perenne* L.)

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Summary

We had previously found differences for seed yield among nine perennial ryegrass cultivars which were not associated with variation for seed weight. To detect the physiological basis of these genetic differences for seed yield, growth analyses were carried out. We related crop development and components of seed yield to seed yield during three years on clay and sandy soil. No significant differences occurred among cultivars for accumulation and partitioning of dry matter or the pattern of tiller production. Seed yield of the cultivars was not associated with ear number or total dry matter yield of the seed crop. Seed yield was more correlated with the number of seeds per unit area than with seed weight. The number of seeds as calculated after harvest from seed yield and seed weight was much lower than the number of seeds as estimated prior to harvest from seed yield components. The number of spikelets differed significantly among the cultivars, but the ranking was different from that for seed yield. The physiological basis of the genetic differences for seed yield is not clear. Implications for breeding perennial ryegrass are discussed.

Introduction

Seed yield in perennial ryegrass is low. Therefore, an extensive study was carried out to identify selection criteria for high seed yielding capacity in perennial ryegrass. We found differences for seed production among nine late-flowering cultivars sown in drilled plots (Elgersma, 1990). Differences in seed yield may result from differences in biomass production, in distribution of assimilates (e.g., harvest index) or both (Wilson, 1984). Seed yield is determined by its components: ear number per unit area, spikelet number per ear, floret number per spikelet, floret site utilization (the percentage of the florets containing a seed) and seed weight. Genetic variation for seed yielding capacity was not associated with that for seed weight (Elgersma, 1990). Therefore, the variation for various other seed yield components, their relative contribution to seed yield and the crop growth pattern were studied to determine the cause of the cultivar differences in seed yielding capacity.

Little is known about genetic variation for the physiological components of seed production in perennial ryegrass. In a one-year trial, Bugge (1987) observed genetic variation for seed yielding capacity among 100 populations. Seed yield was positively correlated with the number of seeds per ear. Correlations between seed yield and ear length, the number of ears and thousand-grain weight were not significant. However, this could be due to the very low nitrogen application which caused incomplete development of spikes and seeds (Bugge, 1987).

Many environmental factors affect seed yield through their effects on yield components. The effects of agronomic treatments on components of seed yield or on crop growth have frequently been studied, usually in one cultivar (e.g., Hampton & Hebblethwaite, 1985; Meijer, 1985; Horeman, 1989; Marshall & Ludlam, 1989).

The effect of an environmental factor on final yield depends on the growth stage(s) in which it operates. Therefore, we tried to minimize developmental variation by choosing only cultivars of the pasture type (Elgersma, 1990). Large differences in seed yield were found among years, which were related to climatic factors. Within years, seed yield and seed weight were higher on clay than on sandy soil and higher in first year crops than in second year crops (Elgersma, 1990).

In this paper, genetic differences for seed yield are related (i) to growth and development of the seed crop and (ii) to components of seed yield.

Materials and methods

Plant material and experimental design

The trials were carried out from 1985 to 1988 at the experimental fields 'Born' and 'Nude' at Wageningen, the Netherlands, on sandy and clay soil, respectively, as described previously (Elgersma, 1990). Nine diploid cultivars of the pasture type were used. Although cultivars with similar ear emergence dates had been chosen, significant differences occurred for time of ear emergence and flowering. 'Perma' was the earliest cultivar followed by 'Semperweide', 'Wendy' and 'Parcour'. 'Trani' and 'Compas' were intermediate, 'Vigor' was rather late, and 'Barenza' and 'Lamora' were the latest. The difference between 'Perma' and 'Barenza' varied from three to ten days in the various environments (Elgersma, 1990).

The seed was sown in the autumns of 1985, 1986 and 1987 at 10 kg ha^{-1} with a row width of 0.25 m in 6-row plots of 1.5 m × 10.5 m.

In 1985, at each location three trials (A, B and C) were established: on sandy soil SA, SB and SC and on clay soil CA, CB and CC (Table 1). Each trial had a randomized block design with four replications and was surrounded by border plots. Thus each trial measured 57 m × 10.5 m. This design allowed application of fertilizer and herbicide to the crop without causing mechanical damage. Plots could be harvested individually and border effects were minimized. Details of the management are described in Elgersma (1990). New trials were established in 1986 and 1987. In total ten trials were sown and studied the same year; in seven trials the second year crop was also studied. Out of these 17, twelve trials were harvested for seed yield and five were used exclusively for growth analyses (Table 1).

Table 1. Overview of trials of first (− 1) and second (− 2) year perennial ryegrass crops. Five trials were exclusively used for growth analyses, twelve were harvested for seed yield (*) and of these, four were also used for determining seed yield components (**).

Year of sowing	Location					
	Sand Observation year			Clay Observation year		
	'86	'87	'88	'86	'87	'88
1985	SA-1* SB-1 SC-1**	SA-2 SB-2* SC-2*		CA-1* CB-1 CC-1**	CA-2 CB-2* CC-2*	
1986		SD-1**	SD-2*			
1987			SE-1**			CE-1* CF-1

Seed yield

Ear emergence date, time of anthesis and the degree of lodging were recorded three times a week. At harvest ripeness (at a moisture content in the seeds of 45 to 50%), the crop of individual plots was cut with a Hege fodder harvester (reciprocating knife mower), put in large jute bags and dried at 25° C to about 11% moisture. The seed was threshed and cleaned. A subsample of about 200 g was cleaned to at least 98% purity and dried at 105° C for 24 hours. Yields were converted to gm^{-2} at 0% moisture. Thousand-grain weight was assessed by weighing three samples of 100 seeds. The number of seeds per unit area was calculated per plot from seed yield and thousand-grain weight (Elgersma, 1990).

Growth analyses

In 1986, in trials SB-1 and SC-1 samples for growth analysis were taken periodically with an interval of about two weeks, starting from April, by cutting all the plants within a 0.75 m^2 quadrant (four rows, 0.75 m length) just above soil surface. Fresh weight was determined immediately after cutting. A subsample of about 25% of the total sample was weighed and dried at 105° C for 24 hours. Dry weight was determined and total dry matter (DM) was calculated per plot and expressed in gm^{-2}.

At four dates also a second subsample was taken, weighed, and separated into dead material, green leaf blades, stems (plus leaf sheaths) and ears where appropriate. Stems and ears were counted. All plant material was then dried to a constant weight. The fraction of generative tillers was obtained by dividing the number of ears by the number of stems. From the ratio of subsample to total sample, dry weight, the number m^{-2} and dry weight m^{-2} of the various components were calculated. In trials SC-1 and CC-1, within a row of each plot 0.20 m was marked, in which tillers and ears were periodically counted.

In 1987, samples for growth analysis in second year crops were taken in trials SA-2 and CA-2. On

Table 2. Summary of the development of perennial ryegrass seed crops and climatic conditions in 1986, 1987 and 1988. The developmental dates are averages of nine cultivars. T indicates mean temperatures (° C) at 1.50 m, Rain total rainfall (mm), and Rad the total global radiation (J cm^{-2}) averaged per day

Crop development	1986	1987	1988
spring development	slow	slow	quick
first crop growth	end March	end March	begin March
date of ear emergence (ee)	June 12	June 10	May 30
date of first anthesis (fa)	June 26	July 2	June 13
date of end of anthesis (ea)	July 14	July 19	June 30
harvest date (hd)	July 30	August 18	July 23
secondary vegetative tillers	no	yes	yes
harvest conditions	good	poor	poor

Period	1986				1987				1988			
	Days	T	Rain	Rad	Days	T	Rain	Rad	Days	T	Rain	Rad
Jan./Feb.		−0.8	123	330		−0.9	65	266		5.0	186	270
March/April		5.3	136	827		6.4	126	1078		6.9	129	1167
May		13.6	68	1761		10.0	82	1495		14.3	38	1220
June		16.7	39	1990		14.0	93	1321		14.9	20	1540
ee-fa	14	19.3	12	2282	22	14.2	35	1386	14	13.8	8	1360
fa-ea	18	19.5	13	2099	17	18.3	62	2050	17	15.3	8	1642
ea-hd	17	16.2	34	1443	21	14.7	85	1159	24	16.0	31	1230

10/6 and 6/7, a 1.0 m^2 quadrant was cut and DM was determined. Component dry weight was obtained as in 1986. Samples of a row of 0.10 m were taken on 22/5, 23/6 and 23/7. They were only used for assessing relative DM partitioning (percent of the total DM) and not for absolute DM, because DM would probably be overestimated due to the small sample. At harvest (11/8), seed weight was determined from a 0.20 m sample. In trial SD-1 per plot 0.40 m was marked. Tillers and ears were counted periodically and prior to the harvest of the whole plot, the marked areas were cut and the material was separated for a growth analysis.

In 1988, growth analyses were carried out in first year crops in trial CF-1. A 1.0 m^2 quadrant was cut on 18/5, 9/6, 28/6 and 19/7, and dry weight of the various components was determined from a subsample of 0.10 m.

Seed yield components

In 1987, in the first year crop (trial SD-1) the spikelets were counted per ear in the marked areas of 0.40 m. In second year crops (trials SA-2 and CA-2), the spikelets were counted in all the ears of the subsamples taken for growth analysis on July 23. Also, in two blocks (18 plots) a row sample of 0.20 m was taken on July 17, about two weeks after anthesis. Florets and seeds were counted in the central spikelet of all ears. The mean number of florets per spikelet was calculated based on all spikelets sampled. For other calculations, only spikelets were taken into account that had at least one floret in which seed set could be observed, i.e., very late spikelets that had not flowered yet were excluded. The percentage of spikelets that could be observed was calculated per plot. Based on these spikelets, the mean number of observed florets per spikelet and the number of seeds were determined and floret site utilization (fsu) was calculated as (seeds/florets) × 100%. In 1988, in trial SE-1 0.10 m was sampled in 2 blocks on July 15, about one month after anthesis. Per plot, observations were made on the number of ears, ear length (measured from the base of the lowest spikelet to the base of the top spikelet), the number of spikelets per ear, the number of florets in the central spikelet (the rudimentary topflorets were not counted) and the number of normally developing seeds. Also, the position of each seed in the spikelet was recorded. Floret site utilization (fsu) was calculated. Compactness of the ear was calculated as the number of spikelets/cm earlength. Per plot, means and ranges were calculated and characters were correlated. On a plot mean basis, analyses of variance were conducted for each character (9 cultivars in two replications) and correlations were calculated.

Results

The environments sampled in this study provided a range of variation in growing conditions: first and second year crops were used, the two locations provided different soil types and the climate varied greatly across the three years (Table 2).

The pattern of crop growth, tillering and partitioning of dry matter

There were no consistent, significant differences among the nine cultivars for accumulation or partitioning of dry matter during 1986, 1987 and 1988. Crop growth in 1986 averaged over cultivars is illustrated in Figs. 1A and 1B. The accumulation of dry matter (DM) of tillers, green leaves, dead materials and ears is presented in Figs. 1C and 1D. Only slight differences occurred among locations. The relative DM partitioning on clay soil during the three years is presented in Fig. 2. Whereas in 1987 the climatic conditions and the crop growth rate were different from those in 1986 (Table 2), the relative DM partitioning was almost similar (Fig. 2). In 1988, the maximum DM yield was already obtained at anthesis and in comparison with 1986 and 1987, the percentage dead materials was high. At ear emergence, leaf DM had already declined to about 15 percent of the total DM. It was then already lower than the percentage dead materials, whereas in 1986 and 1987 this situation was not

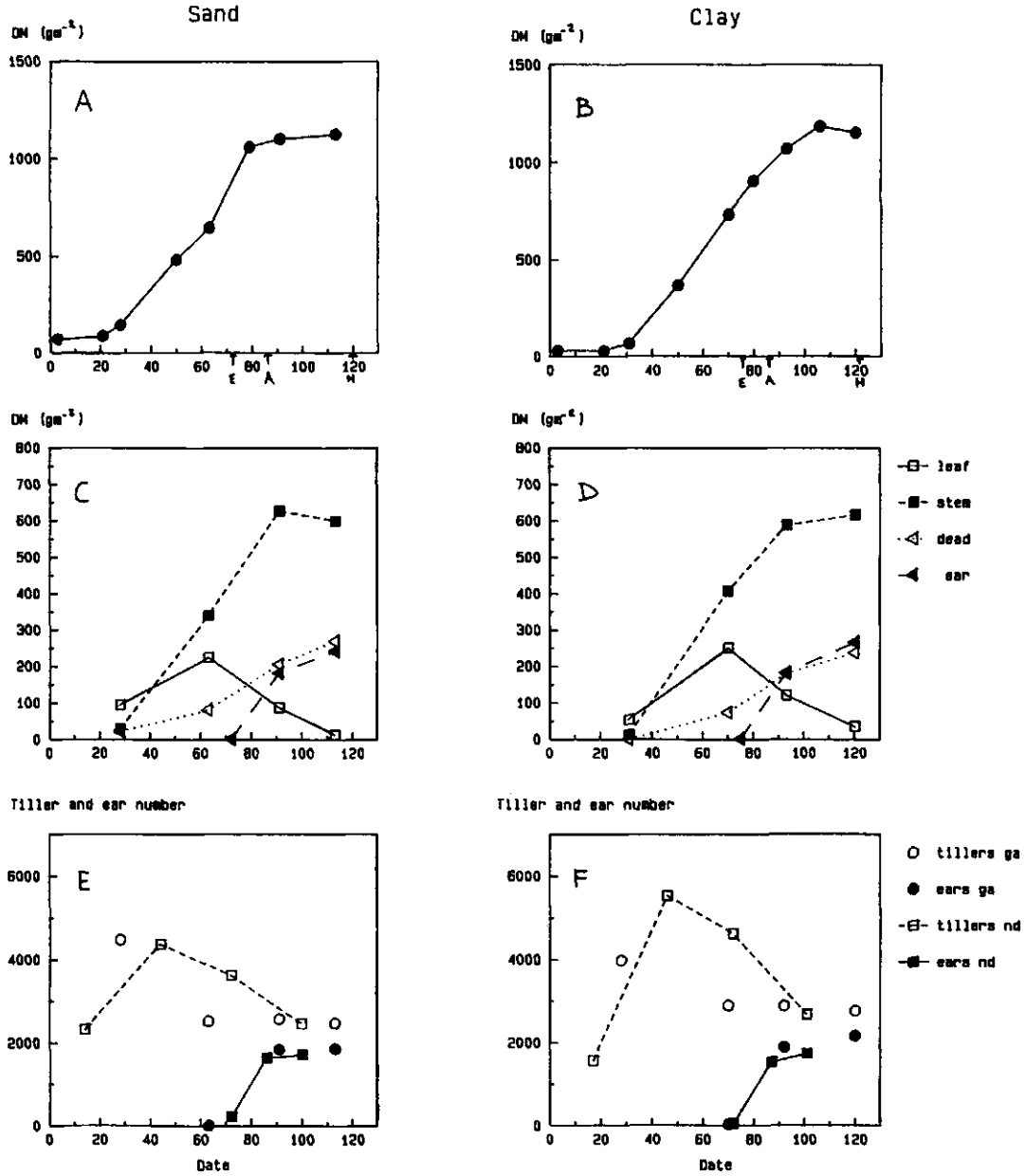

Fig. 1. Total above-ground dry matter (DM) accumulation (Figs. A and B), DM of green leaves, stems, dead materials and ears (Figs. C and D) and tiller and ear numbers m^{-2} resulting from growth analyses (ga) and non-destructive counts (nd) (Figs. E and F) on sandy and clay soil, respectively, averaged over nine perennial ryegrass cultivars in 1986. Dates are expressed in days from April 1st. On the abscissa, E indicates the date of ear emergence, A of first anthesis and H the harvest date.

reached until anthesis. However, in 1988 the distribution of DM at harvest resembled that in 1986 and 1987 (Fig. 2).

The number of tillers revealed no significant differences among cultivars during the three years. In general, the number of tillers increased strongly prior to ear emergence and then decreased dramatically as illustrated for 1986 in Figs. 1E and 1F. The number of generative tillers (ears) increased from ear emergence. No consistent genetic differences for the number of ears were found. Tillers and ear numbers were usually higher on clay than on sandy soil, but 'Semperweide' and 'Wendy' had more tillers and ears on sand than on clay. Initial differences among the cultivars for the weight of individual ears were related to differences in earliness and disappeared at harvest. In 1987, the cultivars did not differ for the number of ears but in 1988 'Compas', 'Perma' and 'Vigor' had significantly more ears than 'Wendy', 'Lamora' and 'Semperweide'.

Seed yield and its components

Summaries of seed production characters are presented per year in Tables 3, 4 and 5. Phenotypic correlations were calculated on a cultivar mean basis between seed yield and other characters (Table 6). Results will be discussed per character.

Harvest index

Seed yield can be considered the product of biomass production and harvest index. The harvest index (hi), calculated as seed DM/total DM, is presented in Table 3. Harvest index was associated with seed yield in 1986 on sandy soil, as the cultivars differed for seed yield but had a similar dry matter production (Table 3). In other environments also, seed yield was positively correlated with harvest index (Table 6).

Seed weight and seed number

Seed yield can be considered the product of the number of seeds and the average seed weight. In most environments, the cultivars differed significantly for thousand-grain weight, but the ranking was different from that for seed yield (Tables 3, 4

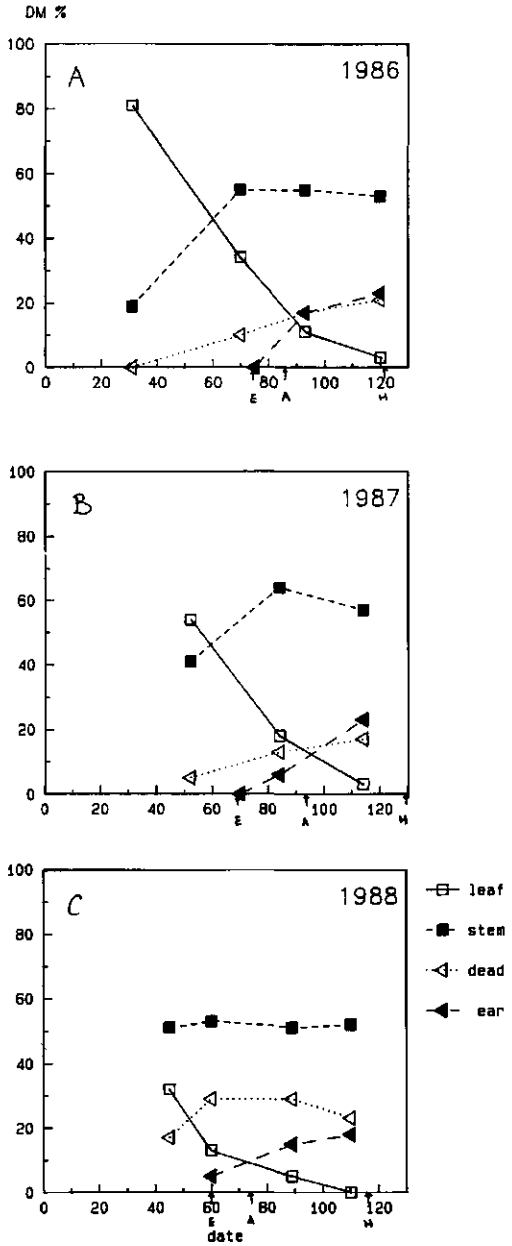

Fig. 2. Relative partitioning of dry matter (DM) over green leaves, stems, dead materials and ears on clay soil, averaged over nine perennial ryegrass cultivars in 1986 (Fig. A), 1987 (Fig. B) and 1988 (Fig. C). Dates are expressed in days from April 1st. E indicates ear emergence, A first anthesis and H harvest date.

and 5). On a cultivar mean basis, no consistent relation was found between thousand-grain weight and seed yield in the various environments (Table 6). The number of seeds per unit area was the only component that was consistently positively correlated with seed yield (Table 6).

Ears

The number of ears did generally not differ significantly among the cultivars and was not associated with seed yield.

Spikelets

The average number of spikelets per ear showed significant differences among cultivars both on clay and on sand in 1987. The number of spikelets was largely the same on both locations. 'Barenza', 'Vigor' and 'Trani' had more spikelets per ear than 'Compas' and 'Semperweide', and 'Wendy' had the lowest spikelet number per ear (Table 4). Also in 1988, 'Trani' and 'Barenza' had significantly more spikelets than 'Wendy' (Table 5). The number of spikelets per ear was not correlated with seed yield (Table 6).

Florets and seeds

In 1987, about two weeks after anthesis in total 2124 spikelets were sampled on sand and 1584 on clay soil in second year crops. In 1988, 1044 spikelets containing over 5000 florets were sampled in first year crops on sandy soil one month after anthesis. The spikelets were checked with a binocular microscope for the presence of normally developing seeds. Wide ranges for the number of florets and seeds per spikelet were observed within plots. In 1987, many florets still contained anthers or the ovaries were too young to determine seed set. On sandy soil, in the early cultivars over 85% of the

Table 3. Seed production traits of first year crops of nine perennial ryegrass cultivars in 1986 on sandy and clay soil

Cultivar	Soil	seed yield (gm^{-2})	thousand grain weight (g)	seed number ($10^4 m^{-2}$)	total dry matter (gm^{-2})	harvest index	ear number (m^{-2})
	Sand						
Trani		140.6	1.45	9.7	1216	0.12	1716
Wendy		127.4	1.46	8.7	1084	0.12	1908
Semperweide		109.6	1.45	7.5	1147	0.10	1866
Vigor		123.7	1.28	9.7	1026	0.12	1776
Parcour		108.6	1.31	8.3	1136	0.10	1919
Perma		117.2	1.45	8.1	1135	0.10	1585
Compas		125.1	1.25	10.0	1059	0.12	2046
Barenza		107.7	1.19	9.0	1010	0.11	1719
Lamora		102.5	1.38	7.4	1285	0.08	2200
Mean		118.0	1.36	8.7	1126	0.10	1859
LSD 5%		14.1	0.08	1.0	213		431
	Clay						
Trani		205.0	1.46	14.0	1124	0.18	2161
Wendy		199.5	1.56	12.8	1094	0.18	1706
Semperweide		183.3	1.58	11.6	1168	0.16	1740
Vigor		190.3	1.39	13.7	1172	0.16	2375
Parcour		169.0	1.47	11.5	1079	0.16	2371
Perma		185.5	1.48	12.5	1071	0.17	2363
Compas		186.5	1.38	13.5	1157	0.16	2379
Barenza		188.0	1.29	14.6	1308	0.14	2243
Lamora		138.7	1.51	9.2	1205	0.12	2248
Mean		182.9	1.46	12.6	1153	0.16	2175
LSD 5%		19.8	0.09	1.5	184		478

spikelets had at least one floret in which seed set could be observed, whereas in the late cultivars this was less than 70%. There were no significant differences for the total number of florets per spikelet (Table 4). In early cultivars, significantly more florets per spikelet could be observed for seed set and thus more seeds were present than in late cultivars, but the seed set itself did not differ among the cultivars. On clay soil the cultivars did not differ for the number of florets or seeds per spikelet or for fsu. In 1988, the development of the seeds was more advanced than in 1987, and all spikelets could be observed. Within each plot, the correlations between ear length, the number of florets per spikelet and the number of seeds per spikelet were positive. Ear length was also positively correlated with the number of spikelets. Compact ears were associated with low numbers of seeds and florets per spikelet. Fsu was not correlated with other characters. Based on plots means, 'Trani' had a high number of florets and seeds per spikelet. In contrast to 1987 (Table 4), in 1988 significant genetic differences were found for fsu on sandy soil (Table 5). In 'Perma' and 'Parcour', some seeds had already shattered and therefore the number of florets and seeds per spikelet are underestimated and fsu might be a little overestimated. 'Lamora' had the lowest fsu. No consistent, significant cultivar differences were found for the number of florets and seeds per spikelet or for fsu (Tables 4 and 5) and these characteristics were not correlated with seed yield (Table 6).

Table 4. Seed production traits of second year crops of nine perennial ryegrass cultivars in 1987 on sandy and clay soil

Cultivar	Soil	seed yield (gm^{-2})	thousand grain weight (g)	seed number ($10^4 m^{-2}$)	spikelets ear^{-1} *	seeds $spikelets^{-1}$ **	floret site utilization (%) **
	Sand						
Trani		90.3	1.27	7.1	22.3	4.7	87
Wendy		89.7	1.43	6.3	18.3	5.2	87
Semperweide		84.9	1.44	5.9	19.7	5.7	81
Vigor		75.7	1.24	6.1	21.4	3.9	88
Parcour		87.9	1.37	6.4	19.4	5.4	81
Perma		81.5	1.39	5.9	20.6	5.5	73
Compas		72.0	1.24	5.8	19.8	4.4	78
Barenza		51.3	1.15	4.5	21.2	4.7	86
Lamora		48.7	1.31	3.7	20.2	4.0	84
Mean		75.8	1.32	5.7	20.3	4.8	83
LSD 5%		10.5	0.05	1.0	2.3	0.8	11
	Clay						
Trani		137.9	1.42	9.7	20.0	5.0	85
Wendy		136.3	1.51	9.1	18.9	4.3	75
Semperweide		124.1	1.54	8.1	19.6	5.1	77
Vigor		110.7	1.35	8.2	21.0	4.0	80
Parcour		139.4	1.45	9.6	21.3	4.9	80
Perma		134.8	1.45	9.3	20.6	4.8	73
Compas		119.9	1.31	9.2	19.5	4.7	81
Barenza		98.2	1.26	7.8	21.8	4.0	70
Lamora		93.8	1.39	6.8	20.9	4.4	73
Mean		121.7	1.41	8.6	20.4	4.6	77
LSD 5%		13	0.08	1.1	1.9	1.3	14

* sampled on July 22.
** sampled on July 17.

Table 5. Seed production traits of first year crops of nine perenial ryegrass cultivars in 1988 on sandy soil

Cultivar	seed yield (gm^{-2})	1000-grain weight (g)	seed number ($10^4 m^{-2}$)	ear length (mm)	spikelets ear^{-1}	florets $spikelet^{-1}$	seeds $spikelet^{-1}$	floret site utilization (%)
Trani	73.8	1.10	6.8	204	24.8	5.5	4.2	77
Wendy	69.6	1.15	6.1	183	21.4	5.4	3.9	74
Semperweide	73.4	1.24	6.0	183	22.4	5.1	3.6	72
Vigor	56.6	1.10	5.3	170	23.0	4.9	3.4	70
Parcour	69.5	1.13	6.5	151	22.8	4.6	3.1	71
Perma	68.6	1.10	6.3	176	22.0	4.6	3.6	80
Compas	53.0	1.00	5.3	154	21.7	4.9	3.7	75
Barenza	44.3	0.98	4.6	178	23.9	4.7	3.7	76
Lamora	33.5	1.12	3.0	178	23.2	5.1	3.3	62
Mean	60.3	1.10	5.5	175	22.8	5.0	3.6	73
LSD 5%	26	0.13	2.8	35	1.5	1.2	0.9	9

Floret position

In Fig. 3 the total number of florets observed at various floret positions within the central spikelet is shown for all cultivars. The number of florets at position 1 equals the total number of sampled ears, thus 'Lamora' had few ears and 'Parcour' many. However, because ear density is very variable, the balance between seeds and empty florets (including aborted seeds) is more interesting (Fig. 3). In all cultivars, most florets contained a seed. At higher floret positions, the number of florets decreased, because some spikelets contained only few florets. In 'Trani', 'Vigor' and 'Lamora' this decline began slowly, indicating that most spikelets had at least three florets. In 'Perma' and 'Parcour', the number of florets at positions 2, 3 and 4 was already lower than that at position 1, indicating that many spikelets with only one, two or three florets were present, probably due to shattering. Especially at flo-

Table 6. Phenotypic correlations between mean values of seed yield and other traits of nine perennial ryegrass cultivars in various environments. Year, soil type and crop production year are indicated.
*: $P \leq 0.05$; **: $P \leq 0.01$; d.f. = 7

	Environment						
Year	'86	'86	'87	'87	'87	'88	'88
Soil	sand	clay	sand	sand	clay	sand	clay
Prod. year	1st	1st	1st	2nd	2nd	1st	1st
Trait							
tgw	0.28	−.16	0.66	0.61	0.64	0.74*	0.53
seeds m^{-2}	0.74*	0.86**	0.87**	0.94**	0.91**	0.96**	0.96**
dry matter	−0.10	−0.19	0.38				0.65
hi	0.81**	0.84**	0.74*				0.70*
ears m^{-2}	−0.34	−0.27	−0.19				0.03
spikelets ear^{-1}				−0.20	−0.48	−0.15	
florets ear^{-1}				0.19	−0.40		
seeds ear^{-1}				0.53	0.45		
florets $spikelet^{-1}$				0.53	−0.16	0.21	
seeds $spikelet^{-1}$						0.34	
fsu				−0.18	0.51	0.56	

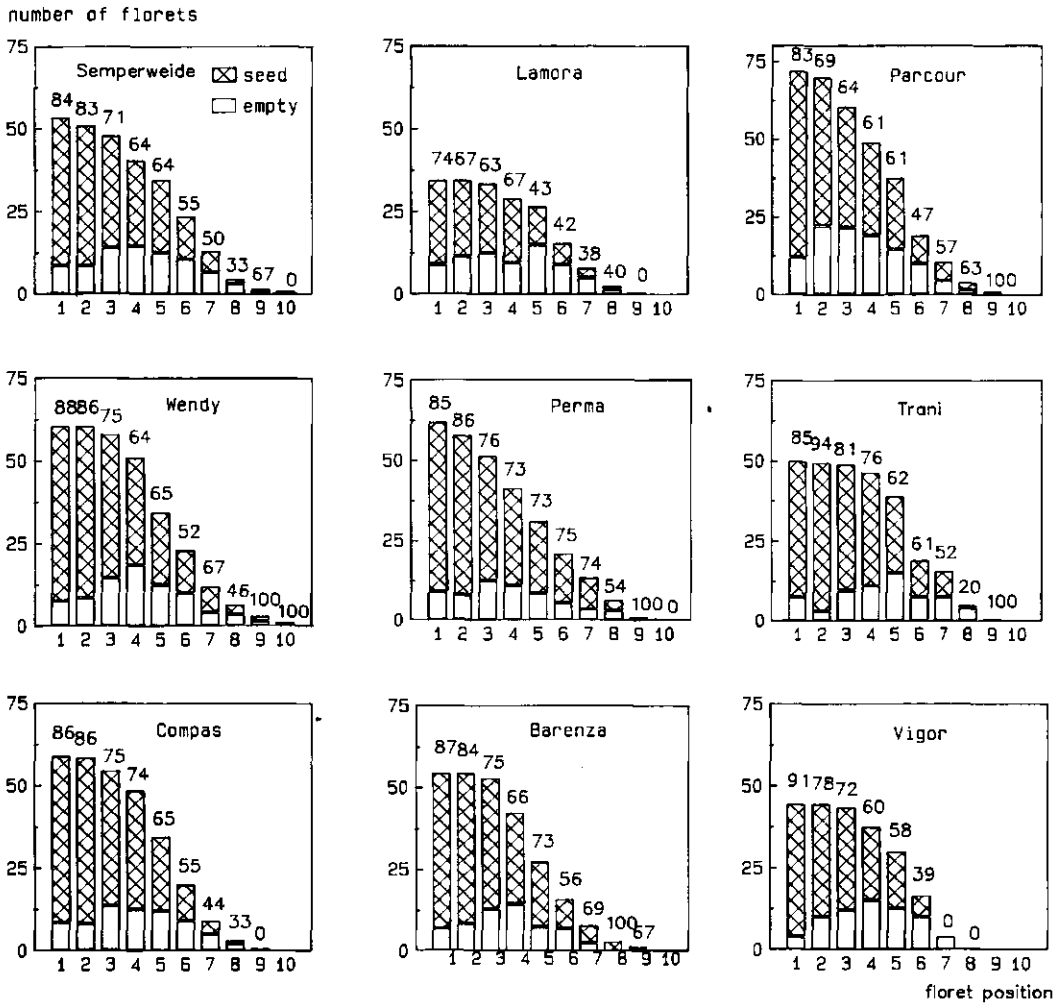

Fig. 3. The total number of florets observed at various floret positions within the central spikelet in a row of 0.20 m in nine perennial ryegrass cultivars in first year crops on sandy soil in 1988. Normally developing seeds and empty florets (including degenerated ovules and aborted seeds) are distinguished. The percentage of florets with a normally developing seed is indicated per floret position.

ret position 7 and higher, only few observations could be made. The absolute number of empty florets increased in central positions. The percentage of normally developing seeds (fsu) is indicated. There was a general decrease in fsu form the lowest to the apical floret positions within the spikelet.

Differences among cultivars

Cultivars can be grouped according to their seed yielding capacity. For example, 'Trani' and 'Wendy' both have a high seed yielding capacity but are quite different: 'Trani' produces many seeds per unit area and 'Wendy' has heavy seeds. 'Trani' has many spikelets per ear and 'Wendy' few, etc. The cultivars 'Wendy' and 'Semperweide' are rather

similar with respect to their relatively early ear emergence, low number of ears, rather heavy tillers and ears, low number of spikelets per ear, average fsu and high seed weight. Nevertheless, 'Wendy' had a high seed yield and 'Semperweide' only an average seed yield. 'Wendy' has a higher harvest index and a higher seed number. 'Lamora' and 'Barenza' both have a low seed yielding capacity. Both cultivars have a late ear emergence date, but 'Barenza' has a very low seed weight and 'Lamora' an average seed weight (similar to that of 'Trani', the highest yielding cultivar). 'Vigor', 'Semperweide' and 'Perma' have a similar average seed yield but a different seed weight, ear emergence date and number of spikelets.

Environmental effects

Seed yield, seed weight and seed number were much higher on clay than on sand. Dry matter production being similar on both locations, the harvest index on clay came out higher than on sand. However, these differences in harvest index could not be explained by differences for growth rate or dry matter partitioning. Soil type affected seed yield much more than seed weight and thus the variation for seed yield between locations was mainly due to different numbers of seeds per unit area. However, the numbers of florets, spikelets and seeds per ear determined in samples were similar on both locations in 1987 and therefore cannot explain the large difference found for seed yield between clay and sandy soil. These components may have been determined in too early a stage of development in 1987.

The large differences for seed yield between years were not related to the total dry matter yield, which was similar across years. The average number of spikelets per ear was higher in 1988 than in 1987 (22.8 versus 20.4), but the number of seeds per spikelet was lower in 1988 (3.6 versus 4.7) (Tables 5 and 4).

Discussion

Genetic variation for seed yield and analysis of components

Previously significant cultivar differences for seed yield were observed and these differences were consistent in various environments. Significant and consistent genetic differences also occurred for seed weight, but the ranking orders of the cultivars for seed yield and seed weight were different (Elgersma, 1990). The present study reveals that genetic differences for seed yielding capacity were not related to differences in crop growth, accumulation and partitioning of DM or lodging behaviour.

Generally, seed yield was related to the number of seeds per unit area, which in turn is determined by the number of ears and by the number of seeds per ear. However, no clear differences for the number of ears were found among the cultivars. Differences for seed yield were neither associated with seed weight per tiller nor with the number of seeds per ear. Significant differences were found among the cultivars for the number of spikelets per ear, but spikelet number was not correlated with seed yield. No clear differences for the numbers of florets and seeds per spikelet and for floret site utilization were found among the cultivars and these characters were not correlated with seed yield.

Seed abortion

According to Hampton (1988), the failure of herbage grasses to realize more than one-tenth of their theoretical seed yield potential is primarily due to poor floret site utilization through abortion of developing seeds. In this view, insufficient assimilates are available to sustain seed growth at all pollinated floret sites.

In the present experiment, fsu generally decreased from the basal to the upper floret of the central spikelet, although non-productive florets were found at all floret positions. Marshall & Ludlam (1989) found in drilled plots that florets within the all positions of the (central) spikelet aborted

ovaries or developing seeds. We also found in spaced plants, that successful fertilization did not decline from the basal to the distal floret. Also along the ear there was no difference between upper, central or basal spikelets. In later stages, distal florets had slightly fewer seeds, but unproductive florets were found at all floret positions (Elgersma & Sniezko, 1988). This random pattern was also found in other grasses by Knowles & Baenziger (1962) and suggests genetical or cytological causes rather than physiological stress.

Marshall & Ludlam (1989) found that about 50% of the florets did not contain a normally developing seed. They suggest that outbreeding species would loose about 50% of their ovules because of genetic defects associated with outbreeding, and in contrast in inbreeding species around 85% of the ovules would survive. However, in this study in various environments on average 73 to 83 percent of the observed florets contained a seed. Moreover, seed set percentages in perennial ryegrass reported by Anslow (1963): 65%, Beddows (1931): 53.9%, Burbidge et al. (1978): 60%, Griffiths & Lewis (1967): 62% and Jenkin (1931): 92.9% do not support this theory.

The number of seeds

The number of seeds per unit area calculated as: number of ears × number of spikelets per ear × number of seeds per spikelet, was three to four times higher than the seed number calculated afterwards from seed yield and thousand-grain weight. The main reason is probably, that all the seeds in a sample are counted, whereas during harvest and cleaning especially small and unripe seeds are lost and other seeds are shed prior to harvest and thus the final seed number is lower. A similar discrepancy between potential yield and realized yield is often encountered (Hebblethwaite et al., 1980). Meijer (1985) determined the number of florets per ear at the end of anthesis and also prior to harvest, when 30% of the initial florets were lost, mainly due to shattering. Fifty-three to 68% of the florets observed prior to harvest contained a seed, but only 40 to 70% of these seeds were recovered after harvest and cleaning. Horeman (1989), too, found that only 25% of the seeds observed prior to harvest were recovered after cleaning, which agrees with our observations.

Potential versus realized seed yield

From the foregoing it is clear, that an important loss of potential yield occurs during and shortly after flowering, when only about 50–80 percent of the florets set a seed. Another major loss occurs during harvest and cleaning, because only about 25–70 percent of the seeds present before harvest are regained.

The loss due to tiller mortality seems less important, because tiller numbers increase dramatically at ear emergence and then decrease again (Figs. 1E, 1F). It is therefore not useful to consider the maximum tiller number in calculations of potential yield. Most of the tillers that are produced late in spring are small and do not survive (Ryle, 1966; Anslow, 1963). The number of fertile tillers (ears) is a more interesting yield component, but we found very variable ear numbers both within and among plots. The average number of ears and the mean seed yield of the cultivars were not correlated.

Environmental effects

Various experiments have been reported on the effect of environmental factors on seed yield, crop growth and yield components. Hampton & Hebblethwaite (1985) found in two years a yield increase following application of a growth regulator, but the yield components responded differently. Yield increases were associated with a higher number of fertile tillers in both years and with an increased number of seeds per spikelet in one year. Other yield components did not change. Horeman (1989) found that the number of spikelets per ear, the number of florets per spikelet and the number of seeds per spikelet did not account for the observed yield increases after fungicide application. In this study, differences between locations must have

been caused by soil type or by soil-weather interaction, because the climatic factors were similar. Soil type did not affect the crop growth rate, the accumulation or the partitioning of dry matter. Seed yield, seed weight and seed number were higher on clay, but the number of ears and other components of yield were not very different. Therefore, the reasons of the higher harvest index and seed yield on clay are not clear.

The relation between seed yield and seed weight was non-linear: seed weight was less affected by environments than seed yield. In general seed size tends to be relatively constant over a range of conditions (Marshall, 1985). The final weight of an individual seed depends mainly on its position within a spikelet (Anslow, 1964). The thousand-grain weight of a crop is calculated after seed cleaning, small seeds are cleaned out and therefore the minimum seed weight depends on the cleaning intensity. This might account for the fact that the average seed weight or thousand-grain weight is rather stable. Consequently, there must be a close relationship between the number of seeds produced per unit area and the yield of seed, which is indeed demonstrated in agronomic studies (Hebblethwaite et al., 1980; Hampton & Hebblethwaite, 1983). We also found within each cultivar high seed numbers in trials with a high yield.

Implications for plant breeding

This study does not clarify the physiological basis of the observed genetic differences for seed yielding capacity. For a plant breeder it will be difficult to predict the seed yielding capacity, because no single seed yield component in drilled plots was identified that is closely correlated to seed yield. Therefore, breeders can probably best assess the yield capacity of new cultivars in separate seed yield trials, comparing the yield with that of standard cultivars. The flowering dates of the cultivars should not differ too much. Plots should not be too small and border effects should be minimized as in this experiment. Agronomic treatment should be according to practice. Plots must be harvested individually at harvest ripeness according to their moisture content and shattering susceptibility. Of course, the harvested seed cannot be used for multiplication because it results partly from pollination by other populations. A safe estimate of the seed production can be obtained in an isolated multiplication, as here the self-incompatibility is expressed. It is recommended to base new cultivars on clones with a similar flowering date and a short flowering period, to minimize spread in flowering and to avoid irregular ripening. Alternatively, increased shattering resistance would allow a delay in harvest until late-emerging ears have fully ripened and consequently increase the recovery of seeds.

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CHAPTER 7

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Heritability estimates of spaced-plant traits in three perennial ryegrass (*Lolium perenne* L.) cultivars

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Summary

Seed yield in perennial ryegrass is low and unpredictable. Spaced-plant traits suitable for indirect selection for total seed yield in drilled plots would be very useful. The objectives of this investigation were to evaluate genetic variation for seed yield components and other traits among clones from three perennial ryegrass cultivars differing in seed yield and their open-pollinated progenies. Per cultivar, a random set of 50 genotypes was cloned and on each genotype seed was generated by open pollination. Clonal ramets of the parents were observed for 17 traits in 1986 at two locations. In 1987 and 1988, parents and progenies were observed as single plants in a randomized complete block design with two replications. There was little cultivar-environment interaction for most traits. The parents differed significantly for almost all traits. Half-sib (HS) families differed for only three to five traits. Broad-sense heritabilities (h^2_b), based on variance components of the parents, were moderate to high; earliness had the highest h^2_b. Narrow-sense heritabilities (h^2_n), based on variance components among HS-families, were low to moderate and mostly not significant; for most traits h^2_n estimates varied between years and cultivars. Flag leaf width and date of first anthesis showed the highest h^2_n. Narrow-sense heritability estimates from parent-offspring regressions (h^2_{nPO}) ranged from non-significant to high, depending on year and cultivar; they were generally higher than the corresponding h^2_n estimates. Generally, h^2_{nPO} was highest for earliness, flag leaf width, ear length and the number of spikelets per ear. Breeding methods that capitalize on additive genetic variance, such as mass selection, should result in improvement for these traits.

Introduction

Perennial ryegrass (*Lolium perenne* L.) is the most important cultivated grass species in Western Europe. Breeding efforts have concentrated on vegetative qualities and many cultivars for forage or turf are now available (Anon., 1990), while less emphasis has been placed on improving seed yield.

Perennial ryegrass is a self-incompatible, wind-pollinated species that is propagated by seed. Commercial seed production occurs in drilled plots. Seed yields are generally rather low and unpredictable (Griffiths *et al.*, 1966; Elgersma, 1985), but there are cultivar differences for seed production (Evans & Muncey, 1977; Nordestgaard & Juel, 1979; Elgersma, 1990). The economics of seed production has become increasingly important for the commercial succes of a new cultivar (van Wijk, 1980). Therefore, plant breeders need selection criteria to ensure a high and stable seed production of new cultivars. Breeders usually start with spaced plants and only after many years of recombination

and selection, advanced populations are tested for seed yielding capacity.

Selection in spaced plants in an early breeding stage might be an effective method to avoid final disappointments due to poor seed production, provided that spaced-plant traits are identified with a high heritability and a high correlation with seed production in drilled plots (indirect selection). Heritability estimates provide an indication of the expected response to selection in a segregating population; as such they are useful in designing an effective breeding program.

Many studies have been reported on spaced plants (e.g., Bean, 1972; Bugge, 1987; Hayward, 1983; Rognli, 1987; Stratton & Ohm, 1989). Bugge (1987) estimated broad-sense heritabilities for some seed yield components, but little is known about narrow-sense heritabilities for spaced-plant traits in perennial ryegrass. Therefore, heritability was estimated for 17 spaced-plant traits in three perennial ryegrass cultivars, which had contrasting seed production in drilled plots. Heritabilities, estimated from clonal parental material (h^2_b), from progeny analyses (h^2_n) and from parent-offspring regressions (h^2_{nPO}), were compared for several years for the three cultivars.

Materials and methods

Materials and methods

Three diploid, late-flowering perennial ryegrass cultivars of the pasture type were used: 'Wendy', 'Barenza' and 'Perma'. When grown in drilled plots these cultivars had a high, low and intermediate seed production, respectively (Elgersma, 1990). From each cultivar, 50 seedlings were taken at random, grown in pots and cloned. In 1985/86, two clonal ramets of each genotype were grown on sand and two on clay soil as part of a larger experiment (a randomized block in two replications). One ramet was kept as a spare. In 1986, each set of 50 genotypes was also grown in a separate rye-isolation. Seeds obtained by open pollination were harvested per genotype and sown immediately in the greenhouse. Two randomly taken seedlings from each progeny were transplanted in Jiffy pots, thus 50 Half-Sib (HS) families consisting of two genotypes were obtained per cultivar. In September 1986, the HS progenies and parental ramets were planted in a field experiment on sandy soil. Parent is equivalent to mother throughout this paper.

The plants were spaced 0.75 × 0.75 m apart. Details of the experimental management are de-

Table 1. Traits and their abbreviations. Traits 1–10 were determined on a plant basis and traits 11–15 on two representative tillers per plant, sampled three weeks after first anthesis. Traits 16–18 were calculated

1.	EE	ear emergence or heading date (days from April 1), when at least three ears are visible
2.	FLL	flag leaf width (mm) at ear emergence
3.	FLW	flag leaf length (mm) at ear emergence
4.	GIE	plant girth (cm) at ear emergence
5.	FA	date of first anthesis (days from April 1)
6.	PH	plant height (cm) when pulled up, after flowering
7.	HD	harvest date (days from April 1)
8.	FT	number of fertile tillers (only in 1986)
9	SYP	seed yield per plant (g)
10.	SYS	seed yield per spike (mg) from ten selected spikes
11.	EL	ear length (mm) from the base of the lowest to the base of the upper spikelet
12.	IN	internode length (mm) from the upper internode to the base of the lowest spikelet
13.	SPK	number of spikelets per ear
14.	FL	number of florets in the central spikelet
15.	S	number of seeds in the central spikelet
16.	FSU	floret site utilization calculated per spikelet as (seeds/florets)*100%
17.	EEFA	days from ear emergence to first anthesis (heading period)
18.	FAHD	days from first anthesis to harvest (ripening period)

scribed elsewhere (Elgersma, 1990). At flowering the plants were tied up to bamboo sticks to prevent loss of tillers and to facilitate harvesting. Ten similar spikes were selected per plant and tied up separately. Harvest ripeness was visually assessed per individual plant according to the colour of the spikes and seeds and to the degree of shattering. Harvesting was carried out per plant with a sickle, the bundle of ten spikes being harvested separately. The spikes were cool-air dried and threshed by hand. The seed was cleaned before weighing.

Following seed harvest in 1987, the stubbles were cut. After regrowth, tillers of the progenies were dug out. Spare ramets of the parental genotypes were taken. The previous experiment was repeated in 1987/88. Thus parents were observed in 1986 on sand and clay and in 1987 and 1988 on sand (four environments) and progenies were observed in 1987 and 1988 on sand. Table 1 shows the traits assessed.

Statistical analyses

Models for analyses of variance

Data were analysed for each of the three cultivars separately. In 1986, per location the 50 parents were randomized within each of two blocks. In 1987 and 1988, 50 parents and 50 progenies were individually randomized as single plants within each of two blocks. The statistical design utilized for a single years' data is a randomized block, consisting of 100 entries, in two replications.

Initially a model with g genotypes observed in b blocks was used:

$$\underline{y}_{ij} = \mu + \underline{g}_i + \underline{b}_j + \underline{e}_{ij} \qquad \text{(Model I)},$$

where $\underline{y}_{ij}$ is the observed phenotypic mean value of genotype i (i = 1...g; g = 50) in block j (j = 1...b; b = 2) and μ, $\underline{g}_i$, $\underline{b}_j$ and $\underline{e}_{ij}$ represent the overall population mean, the effect of the i^{th} genotype, the effect of the j^{th} block and the mean random error of the i^{th} genotype in the j^{th} block, respectively. For each trait the block effect was tested according to model I (Table 2), but it was usually not significant. Since neither a block effect nor a possible genotype – block or family – block interaction was considered to be of primary interest, blocks were ignored in further analyses (Bos, 1981). The observations on the two replications were pooled and a model with g genotypes, each observed on r plants (ramets) was used:

$$\underline{y}_{ij} = \mu + \underline{g}_i + \underline{e}_{ij} \qquad \text{(Model II)},$$

with i = 1...g, j = 1...r, g = 50 and r = 2.

For the offspring a model with g HS-families, each observed on r plants, was used:

$$\underline{y}_{ij} = \mu + \underline{g}_{i\ (\text{between families})} + (\underline{g}_{ij\ (\text{within families})} + \underline{e}_{ij}),$$

where $\underline{g}_i$ is the effect of the i^{th} family and $\underline{g}_{ij}$ is the effect of the i^{th} family and the j^{th} plant, with i = 1...g, j = 1...r, g = 50 and r = 2. The residual error contains both genetic variation within families and environmental variance.

For parents and half-sib families the analysis of variance (ANOVA) according to model II was used (Table 2).

Table 2. Two models with analyses of variance and expectations of mean squares

Model I		
Source of variation	Df	Expected mean squares
Blocks (B)	b-1	$\sigma^2_e + g\sigma^2_b$
Genotypes (G)	g-1	$\sigma^2_e + b\sigma^2_g$
Residual (R)	(b-1)(g-1)	σ^2_e
Model II		
Source of variation	Df	Expected mean squares
Genotypes (G)	g-1	$\sigma^2_e + r\sigma^2_g$
Residual (R)	g(r-1)	σ^2_e

Broad- and narrow-sense heritability

Broad-sense (h^2_b) and narrow-sense (h^2_n) were calculated per environment based on parental means and family means, respectively. Estimates of genetic variance components from clonal analyses

were used to calculate h^2_b (Burton & DeVane, 1953) and estimates of genetic variance components from progeny analyses were used to calculate h^2_n (Nguyen & Sleper, 1983a) as follows:

$$h^2_b = \sigma^2_{gP}/(\sigma^2_{gP} + (\sigma^2_{eP}/r))$$
$$h^2_n = \sigma^2_{gF}/(\sigma^2_{gF} + (\sigma^2_{eF}/r))$$

where, σ^2_{gP}, σ^2_{gF} = variance components due to parents and families, respectively; σ^2_{eP}, σ^2_{eF} = residual mean squares in the ANOVA for parental clones and in the ANOVA for HS-families when calculating h^2_b and h^2_n, respectively; and r = number of replications (here equal to two).

The genetic variation among HS-families (σ^2_{gF}) measures additive genetic variation (1/4 σ^2_a) under the following assumptions: no epistasis, the parental population is in linkage equilibrium, the parents form a random sample from a reference population and the parents produce offspring after random mating. Epistasis is considered to be absent although this cannot be verified. The three last assumptions appear to be fulfilled.

Parent-offspring regression

The linear regression coefficient of HS-progeny values on their parental values is multiplied by two to obtain an estimate of narrow-sense heritability (h^2_{nPO}) (Kempthorne, 1957; Falconer, 1981).

Averages per parent and HS-family were calculated per environment and these form the basis for offspring-parent regressions. In 1987 and 1988, parents and progenies were included in the same blocks (completely randomized). However, potential bias might occur due to non-genetic covariances between parents and offspring (Vogel *et al.*, 1980). This bias can be removed by regressing progeny means on parental means evaluated under different locations *and* years (Casler, 1982). Therefore, offspring values obtained on sandy soil in 1987 and 1988 were also regressed on parental values obtained on clay in 1986, to obtain non-biased estimates.

Results

Mean values

Table 3 shows the mean values for 17 spaced-plant traits and significances of genotypic variation between parental clones and HS-families of the three cultivars in various environments.

Comparison of environments

Mean values of parents and progenies differed in the various environments. The parents differed at both locations (in 1986): on sand all cultivars had longer flag leaves and ears; on clay they had more tillers with longer internodes, more spikelets and florets and a much higher seed yield per plant. The parents also differed over the three year tested on sandy soil. In 1986 the plants were late and had a short ripening period and in 1988 they were early and had a long ripening period. The heading period was also shortest in 1986 but longest in 1987. Flag leaf width and length, plant girth, seed yield per spike, the number of florets and seeds per spikelet and floret site utilization declined from 1986 to 1987 and from 1987 to 1988 in all cultivars. Seed yield per plant was also highest in 1986, but lowest in 1987. Plant height, internode length and the number of fertile tillers were largest in 1987. Ears were shortest in 1988 and the number of spikelets per ear was highest in 1988 and lowest in 1987. These results were found in all cultivars.

Mean values of the HS-families also differed in the two years: in 1988, the offspring means were smaller and plants were earlier than in 1987 for all traits except spikelet number and ripening period, which were larger in 1988. Again, this was irrespective of cultivars.

Thus, parental means and family means varied in a similar way in 1987 and 1988 for all traits except seed yield per plant: parents had a higher seed yield per plant in 1987 than in 1988 and offspring yielded more in 1988 than in 1987.

Comparison of cultivars

Mean values of parents and progenies differed in the three cultivars.

'Barenza' was on average the latest cultivar and

Table 3. Mean values (M) and genotypic components of variance (σ^2_g) for 17 traits in spaced plants of 50 parental genotypes and their HS-families within three perennial ryegrass cultivars over three years. Population (**Pop**) is abbreviated and **PS** indicating Parents on Sandy soil, **PC** for Parents on Clay soil, **P** for Parents and **O** for Offspring. For abbreviations of traits see Table 1

Trait	cv. 'Wendy'								cv. 'Perma'								cv. 'Barenza'							
	1986			1987			1988		1986			1987			1988		1986			1987			1988	
	Pop	M	σ^2_g[1]	Pop	M	σ^2_g	M	σ^2_g	Pop	M	σ^2_g	Pop	M	σ^2_g	M	σ^2_g	Pop	M	σ^2_g	Pop	M	σ^2_g	M	σ^2_g
EE	PS	69	35***	P	71	68***	57	50***	PS	69	31***	P	72	54***	58	28***	PS	75	30***	P	79	57***	64	53***
	PC	68	37***	O	68	9	56	0[2]	PC	68	26***	O	67	1	57	6	PC	74	29***	O	74	15	62	5
FLW	PS	7.6	5***	P	7.3	5***	6.9	5***	PS	7.4	5***	P	7.1	4***	6.6	4***	PS	7.9	7***	P	7.7	8***	6.9	5***
	PC	7.3	5***	O	7.8	3*	7.1	27*	PC	7.2	4***	O	7.9	2*	7.0	0	PC	7.5	4***	O	8.6	5**	7.4	2
FLL	PS	244	763***	P	222	1153***	204	1416***	PS	249	775**	P	239	1259***	206	1177***	PS	244	863***	P	232	2348***	170	436*
	PC	212	728***	O	249	143	210	639*	PC	216	1068***	O	258	415*	226	0	PC	196	903***	O	256	398	185	13
GIE	PS	127	256***	P	100	262***	98	289***	PS	131	204***	P	106	139***	94	66	PS	144	119**	P	116	492***	108	86*
	PC	128	95**	O	125	3	100	31	PC	130	68**	O	124	76*	99	7	PC	140	107***	O	131	0	113	11
FA	PS	85	13***	P	94	27***	78	27***	PS	85	6***	P	95	11***	79	14***	PS	87	9***	P	99	27***	82	29***
	PC	84	8***	O	93	3*	77	3	PC	85	6***	O	93	1	77	5*	PC	87	10***	O	96	5	81	9*
PH	PS	70	20*	P	78	83***	73	67***	PS	74	34**	P	79	108***	72	51***	PS	70	35**	P	78	95***	73	52***
	PC	74	26***	O	87	16	74	0	PC	74	38***	O	85	0	73	16	PC	70	29**	O	84	18	76	0
FT	PS	310	3078*	P	354	12458***	–	–	PS	370	2636*	P	385	7321***	–	–	PS	359	331	P	428	9350***	–	–
	PC	453	5310*	O	475	0	–	–	PC	503	2335	O	477	0	–	–	PC	512	5465*	O	491	0	–	–
SYP	PS	40	109***	P	19	122***	24	56	PS	41	196***	P	13	91***	17	57**	PS	37	84**	P	15	118***	16	55***
	PC	74	182**	O	30	0	25	0	PC	69	320***	O	20	82*	19	48*	PC	65	326***	O	18	18	15	0
SYS	PS	190	9*	P	120	2***	40	2	PS	170	2***	P	80	9***	20	0	PS	160	2***	P	80	2**	1	0*
	PC	210	2**	O	120	7	30	2*	PC	200	3***	O	80	1	30	0	PC	190	29***	O	90	0	2	0
IN	PS	174	591*	P	224	1253**	218	917***	PS	165	864**	P	223	888***	204	603***	PS	164	411*	P	238	844	227	554**
	PC	221	521***	O	245	1280***	212	17	PC	218	403**	O	245	98	208	140	PC	218	536***	O	244	5	230	149
EL	PS	257	503**	P	262	1117***	230	816***	PS	254	894***	P	250	1458***	219	687***	PS	238	850***	P	252	1183***	216	598***
	PC	249	658***	O	283	104	236	0	PC	243	641***	O	267	274	218	63	PC	233	315*	O	268	416	214	110
SPK	PS	23.0	9.1***	P	22.3	5.7***	24.3	7.2***	PS	23.3	6.9**	P	23.2	3.8***	26.4	6.9***	PS	25.8	7.5***	P	24.0	6.0***	28.3	3.3**
	PC	23.7	6.0***	O	22.7	1.8*	24.7	0.5	PC	25.0	5.8***	O	23.6	1.8	25.7	0	PC	26.3	7.2***	O	24.3	0	27.3	0
FL	PS	10.5	0.9**	P	9.5	2.2***	8.1	0.8*	PS	9.7	2.0***	P	8.9	1.3***	8.1	0.9**	PS	9.2	3.1***	P	9.1	1.7***	7.5	1.0**
	PC	10.3	0.9**	O	10.8	0.0	8.7	0.4	PC	10.5	1.6***	O	9.8	0.1	8.4	0.5*	PC	9.8	2.1***	O	9.8	0.5	7.6	0.3
S	PS	8.2	1.5**	P	6.5	2.4***	5.5	2.3*	PS	7.1	2.0**	P	6.5	2.3***	5.6	0.6	PS	7.4	4.0***	P	6.7	1.9*	5.0	0.8
	PC	7.6	1.9***	O	7.4	0.4	6.2	2.0***	PC	7.6	2.7***	O	7.1	0.8	5.7	2.3*	PC	7.3	2.3***	O	7.2	1.3*	5.0	0.7
FSU	PS	78	1***	P	68	2***	66	1	PS	73	2***	P	72	2**	67	0	PS	78	2***	P	73	1*	66	2**
	PC	73	1***	O	69	0	68	2*	PC	72	2***	O	72	0	66	1	PC	73	1*	O	71	1	64	3**
EEFA	PS	16	8***	P	23	17***	21	3*	PS	16	9***	P	23	17***	20	4***	PS	13	8***	P	20	12***	18	5**
	PC	16	11***	O	25	2	21	0	PC	16	9***	O	25	2	21	1	PC	13	6***	O	22	3	19	0
FAHD	PS	28	2	P	29	10**	34	3	PS	27	4**	P	29	10**	32	12***	PS	27	4***	P	30	18***	33	8***
	PC	31	7***	O	30	0	34	2	PC	28	3**	O	30	0	31	2	PC	30	4***	O	28	9*	33	6*

***, ** and * indicate significance of the mean square associated with the variance component at $P \leq 0.001$, 0.01 and 0.05, respectively, based on the ANOVA F-tests.

[1] σ^2_g indicates the variance component due to parents (σ^2_{gP}) for PS, PC and P and the variance component due to HS-families (σ^2_{gF}) for O, respectively.

[2] every 0 indicates a negative component estimate.

Table 4. Broad-sense (h^2_b) and narrow-sense heritabilities based on sib-analyses (h^2_n) and on parent-offspring regressions (h^2_{nPO}) of 17 spaced-plant traits in three perennial ryegrass cultivars over three years. The h^2_{nPO} estimates obtained by regression offspring means from 1987 and 1988 on parental means from 1986 on clay, are printed in italics

cv. 'Wendy' Trait	h^2_b				h^2_n		h^2_{nPO}			
	'86 sand	'86 clay	'87	'88	'87	'88	'87		'88	
EE	**0.92**	**0.97**	**0.96**	**0.94**	0.34	0	**0.60**	***0.88***	**0.62**	***0.68***
FLW	**0.77**	**0.72**	**0.64**	**0.67**	**0.42**	**0.43**	**0.92**	***0.95***	**1.04**	***0.97***
FLL	**0.63**	**0.59**	**0.74**	**0.71**	0.12	**0.47**	**0.54**	*0.50*	**0.89**	***1.06***
GIE	**0.66**	**0.52**	**0.65**	**0.82**	0.02	0.15	0.00	*0.00*	0.04	*0.00*
FA	**0.90**	**0.91**	**0.92**	**0.92**	**0.36**	0.27	**0.44**	***0.96***	**0.88**	***1.24***
PH	**0.46**	**0.63**	**0.68**	**0.66**	0.28	0	**0.58**	*1.20*	0.14	*1.02*
FT	**0.44**	**0.46**	**0.81**	–	0	–	0.18	*0.54*	–	–
SYP	**0.61**	**0.51**	**0.77**	**0.42**	0	0	**0.48**	*0.24*	0.38	*0.20*
SYS	**0.38**	**0.57**	**0.82**	0.38	0.30	**0.40**	0.33	*0.10*	0	*0*
IN	**0.50**	**0.66**	**0.56**	**0.70**	**0.59**	0.02	**0.60**	*0.48*	0.48	*0.24*
EL	**0.54**	**0.69**	**0.76**	**0.82**	0.12	0	0.44	***0.82***	**0.58**	***0.76***
SPK	**0.74**	**0.75**	**0.80**	**0.77**	**0.43**	0.08	**0.70**	***0.52***	**0.74**	*0.50*
FL	**0.56**	**0.59**	**0.72**	**0.52**	0.03	0.33	0.30	*0.54*	0.06	***0.80***
S	**0.57**	**0.63**	**0.75**	**0.79**	0.17	**0.77**	0.09	*0*	0.14	*0*
FSU	**0.69**	**0.61**	**0.69**	0.53	0.20	**0.64**	0	*0*	0	*0*
EEFA	**0.78**	**0.88**	**0.87**	**0.50**	0.17	0	**0.50**	*0.76*	0	*0.30*
FAHD	**0.27**	**0.73**	**0.58**	0.34	0	0.35	0.02	*0.37*	**0.68**	***0.46***

cv. 'Perma' Trait	h^2_b				h^2_n		h^2_{nPO}			
	'86 sand	'86 clay	'87	'88	'87	'88	'87		'88	
EE	**0.96**	**0.96**	**0.95**	**0.90**	0.07	0.29	**0.54**	***0.62***	**0.64**	***0.62***
FLW	**0.69**	**0.76**	**0.67**	**0.62**	**0.40**	0.00	0.54	*0.58*	0.48	***0.76***
FLL	**0.56**	**0.77**	**0.78**	**0.63**	**0.40**	0	0.34	***0.80***	0.06	***0.64***
GIE	**0.68**	**0.53**	**0.60**	0.37	**0.40**	0.04	0.28	*0.38*	0.52	*0.42*
FA	**0.79**	**0.88**	**0.85**	**0.80**	0.14	**0.45**	**0.48**	***0.54***	**0.58**	***1.10***
PH	**0.55**	**0.73**	**0.81**	**0.68**	0	0.26	0.30	***0.80***	0.28	*0.36*
FT	**0.44**	**0.32**	**0.72**	–	0	–	0.14	*0.04*	–	–
SYP	**0.74**	**0.69**	**0.81**	**0.59**	**0.43**	**0.46**	0.32	***0.34***	**0.88**	***0.30***
SYS	**0.61**	**0.72**	**0.61**	0	0.36	0.34	0.16	*0.04*	0.00	*0.02*
IN	**0.46**	**0.55**	**0.62**	**0.71**	0.08	0.18	0.08	*0.50*	0.46	***0.86***
EL	**0.62**	**0.66**	**0.87**	**0.83**	0.24	0.12	**0.60**	***0.88***	**0.60**	***0.40***
SPK	**0.58**	**0.77**	**0.74**	**0.76**	0.35	0	0	*0.40*	**0.68**	***0.50***
FL	**0.72**	**0.73**	**0.66**	**0.61**	0.06	**0.41**	**0.44**	***0.52***	0.38	*0.02*
S	**0.63**	**0.68**	**0.72**	0.25	0.34	**0.63**	0.08	*0.36*	0.56	*0.34*
FSU	**0.73**	**0.69**	**0.65**	0.06	0.14	0.25	0.18	*0.28*	0.80	*0.18*
EEFA	**0.80**	**0.81**	**0.89**	**0.68**	0.16	0.19	**0.50**	***0.60***	0.24	***0.50***
FAHD	**0.54**	**0.53**	**0.58**	**0.74**	0	0.14	0.02	*0.11*	0.06	*0.78*

cv. 'Barenza' Trait	h^2_b				h^2_n		h^2_{nPO}			
	'86 sand	'86 clay	'87	'88	'87	'88	'87		'88	
EE	**0.94**	**0.96**	**0.95**	**0.95**	0.36	0.22	**1.00**	***1.20***	**0.64**	***0.92***
FLW	**0.77**	**0.67**	**0.75**	**0.63**	**0.50**	0.30	**0.72**	***0.90***	**0.59**	*0.54*
FLL	**0.69**	**0.72**	**0.83**	**0.43**	0.15	0.01	**0.42**	***0.56***	0.40	*0.36*
GIE	**0.53**	**0.82**	**0.92**	**0.50**	0	0.07	0.04	*0*	0.28	*0*
FA	**0.93**	**0.92**	**0.92**	**0.94**	0.36	**0.45**	**0.90**	***1.16***	**0.96**	***1.26***
PH	**0.63**	**0.52**	**0.79**	**0.68**	0.33	0	**0.40**	*0.00*	0.00	*0.08*
FT	0.06	**0.44**	**0.75**	–	0	–	0.06	*0*	–	–
SYP	**0.50**	**0.79**	**0.86**	**0.64**	0.20	0	**0.80**	*0.06*	0.24	*0.08*
SYS	**0.62**	**0.96**	**0.75**	**0.48**	0.31	0.14	0.48	*0.20*	**0.77**	*0.08*
IN	**0.41**	**0.60**	0.53	**0.53**	0.00	0.15	**0.50**	***0.62***	0.32	*0.06*
EL	**0.70**	**0.49**	**0.78**	**0.70**	0.36	0.23	0.46	***0.85***	**0.56**	***0.62***
SPK	**0.88**	**0.77**	**0.74**	**0.53**	0	0	**0.42**	***0.43***	0.14	*0.18*
FL	**0.85**	**0.77**	**0.68**	**0.58**	0.33	0.22	0.36	*0.36*	0.17	*0.24*
S	**0.78**	**0.60**	**0.53**	0.45	**0.45**	0.41	0.14	*0*	0.36	*0.02*
FSU	**0.67**	**0.46**	**0.53**	**0.56**	0.35	**0.79**	0.10	*0.01*	0.56	*0.04*
EEFA	**0.83**	**0.77**	**0.70**	0.59	0.22	0	**0.60**	*0.64*	0.14	*0.24*
FAHD	**0.79**	**0.62**	**0.65**	**0.67**	**0.43**	**0.44**	0	*0.56*	**0.67**	*0.38*

had the shortest heading period. 'Barenza' also had the largest girth at ear emergence, the widest flag leaves, most fertile tillers ('Wendy' fewest), most spikelets ('Wendy' fewest) and fewest florets per spikelet ('Wendy' most). 'Wendy' had the highest seed yield per plant and per spike, the longest ears and most seeds per spikelet. These differences were found in all environments for both parents and offspring. Thus 'Wendy' and 'Barenza', with a high and low seed yield per plot, respectively, showed also the largest contrast on a spaced-plant basis.

Parents versus offspring

In 1987, mean values of the offspring exceeded parental means for almost every trait and ears of the offspring emerged and flowered slightly earlier. This was found in all cultivars. In 1988, offspring and parental means were about the same, although in 'Wendy' offspring means were still slightly larger for most traits. In both 1987 and 1988 the ranges of the traits measured in the offspring (not shown) were similar or slightly smaller than the parental ranges.

Genotypic variation and heritabilities

For almost every trait and for each cultivar significant differences were present among the 50 parental genotypes in the four environments (Table 3). In contrast, no significant difference among HS-families was found for most traits. In 'Wendy', σ^2_{gF} was significant for only four traits (out of 17) in 1987 and for five traits (out of 16) in 1988. In 'Perma' it was significant for four traits and in 'Barenza' for only three traits in both years, respectively. Moreover, σ^2_{gF} was significant for different traits in 1987 and in 1988.

The variance component estimates from the progeny analyses were smaller than those from the parental analyses in both years (Table 3). This is expected, since the progeny component measures only the additive portion of the total genetic variation. Heritability estimates for those traits where no significant genetic variation was detected in the parent and progeny ANOVA are meaningless. Therefore, significant values (Table 3) are printed bold in Table 4. The heritability estimates from the parent-offspring regression are printed bold, if the regression coefficient exceeds twice its standard error.

Most h^2_b values were moderate to high in the three cultivars. In all cultivars, estimates were very high for earliness (dates of ear emergence and first anthesis) in all environments.

The h^2_n values were low to moderate. Within a cultivar, estimates were rarely significant for the same trait in both 1987 and 1988. The three cultivars generally had different traits with a significant h^2_n; the h^2_n estimates were only significant in all cultivars for flag leaf width and date of first anthesis.

The h^2_{nPO} values were significant for more traits, although the parent-offspring analyses showed generally much scatter around the fitted regression line and the percentage variance in the offspring accounted for by the parents was low. In 1987 and 1988 biased estimates of h^2_{nPO} were significant for nine and seven traits in 'Wendy', for five and five traits in 'Perma' and for nine and six traits in 'Barenza', respectively. 'Wendy' and 'Barenza' showed largely the same pattern. In all cultivars, heritability estimates for earliness, seed yield per plant, ear length, spikelets per ear and heading period were significant in one or both years. Non-biased estimates of h^2_{nPO} were significant for eight to nine traits in 'Wendy' and 'Perma' in both years but in 'Barenza' for only seven and three traits in 1987 and 1988, respectively. Estimates for earliness and ear length were significant for all cultivars in both years and for flag leaf length and width and spikelet number, in one or both years.

Thus, in general both biased and non-biased h^2_{nPO} estimates were significant for flag leaf width, earliness, ear length and the number of spikelets per ear.

Discussion

The most convenient way to assess heritability is to use clonal material, which is easily obtained in grasses by splitting a plant into ramets of one or more tillers. In this manner traits can be assessed in different environments. There was little cultivar – environment interaction for most traits on the basis of cultivar means: for example genotypes from

'Barenza' and their progenies matured later in all environments than those from the other cultivars and parents and progenies of all cultivars matured later in 1987 than in other years.

Based on clonal parental material, variance components can be estimated and used to calculate h^2_b (Burton & DeVane, 1953). When clonal propagation is practiced, h^2_b indicates the response to clonal selection. However, perennial ryegrass is generatively propagated. Therefore, h^2_b overestimates the response to selection as it includes non-additive effects. In this study, h^2_bs were generally moderate to high. They were very high for earliness, which is not surprising (Cooper, 1959). Bugge (1987) found in perennial ryegrass h^2_bs for ear length, numbers of spikelets per ear and florets per spikelet, seed yield per plant and fertile tiller number of 0.92, 0.83, 0.71, 0.64 and 0.49 (based on two years and three ramets/genotype). These values are comparable with the present data. Progeny testing provides estimates of narrow-sense heritability, which are more realistic for a crop with generative multiplication. Here, estimates for h^2_n were indeed considerably lower than those for h^2_b.

In this study, h^2_n was estimated on a family mean basis. It is also possible to estimate h^2_n on the basis of individual plants within families. However, when the families are not cloned, the within-family genetic variation and the environmental variation are confounded and the latter is expected to be large (Aastveit & Aastveit, 1990). A theoretical possibility would be to substitute the σ^2_{eP} from the parental analyses for the environmental variation of the half-sibs and then estimate the genetic variation within HS-families (P. Stam, pers. comm.). Alternatively, h^2_n could be estimated directly from the sib-analyses as $4\sigma^2_{gF}/(\sigma^2_{gF} + \sigma^2_{eF})$, because $4\sigma^2_{gF}$ is an estimate of σ^2_a and σ^2_{eF} estimates $3/4\ \sigma^2_a + \sigma^2_d + \sigma^2_e$ and thus $(\sigma^2_{gF} + \sigma^2_{eF})$ equals $\sigma^2_p = \sigma^2_a + \sigma^2_d + \sigma^2_e$ (I. Bos, pers. comm.). However, such estimates often resulted in heritability values > 1. Since in this study only two plants were present per family, within-family variation would be based on a minimal sample (Aastveit & Aastveit used 20 plants per family), and therefore heritability estimates based on individual plants within progenies are not presented in this paper.

Parent-offspring regression is commonly used in forage grass breeding. The most efficient design is to have as many families as possible and to measure only one plant per family (Falconer, 1981). According to Nguyen & Sleper (1983a) the regression method provides a more satisfactory estimate of narrow-sense heritability than does the sib analysis method. There was little agreement between h^2_n and h^2_{nPO}. The h^2_n values were not significant for most traits, with the exception of flag leaf width and date of first anthesis. For these two traits a considerable portion of the total genetic variation is additive. However, the h^2_{nPO} values were significant for more traits. Nguyen & Sleper (1983b) and Wofford & Baltensperger (1985) also found little agreement between narrow-sense heritabilities from sib-analysis and parent-offspring regression for seed yield traits in *Festuca arundinacea* and for turfgrass traits in *Cynodon dactylon*, respectively.

Regression of offspring means on parental means from the same trial (biased) resulted in about half of the 99 comparisons in higher heritability estimates than regression on parental means from another environment (non-biased), although the first were expected to be inflated (Casler, 1982). Apparently, the environmental covariances between parents and offspring were of minor importance in this study.

A perennial ryegrass plant can be considered a population of individual tillers with a limited lifespan. Although perennial ryegrass is rejuvenated by cloning, still the physiological age of a plant increases and also the chance for diseases. The best comparison would therefore be between parents in 1986 and progenies in 1987, when both populations were in the year following germination. Thus the relationship offspring 1987 – parents 1986 is expected to be better than the relationship offspring 1988 – parents 1986. Indeed, for most traits the non-biased heritability estimates were higher in 1987 than in 1988 in 'Barenza', but not in the other cultivars.

Wofford & Baltensperger (1985) found significant variation among clones and HS-families for 13 out of 18 turfgrass traits in *Cynodon dactylon*. Aastveit & Aastveit (1990) found in *Festuca pratensis* highly significant additive variation for earliness and dry matter yield, both vegetative traits. In this

study both h^2_{nPO} estimates were significant for earliness, ear length, flag leaf width and the number of spikelets per ear in all cultivars. These traits could possibly be used for selection. Earliness, flag leaf width and ear length are registration characters; the number of spikelets per ear is a seed yield component. Most seed yield components (fertile tillers, seed yield per spike, numbers of florets and seeds and floret site utilization) and seed yield per plant are not promising for selection in spaced plants.

When seed yield per plot is predicted from a trait assessed in spaced plants, there may be interaction due to plant spacing in addition to the low heritability of that trait and to the lack of correlation between that trait and total seed yield per plot. Therefore, the progeny should be observed both as spaced plants and in drilled plots. The performance of the HS-families in drilled plots is currently studied.

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CHAPTER 8

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Spaced-plant traits related to seed yield in plots of perennial ryegrass (*Lolium perenne* L.)

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Summary

Seed yield in perennial ryegrass is low and selection criteria for high seed production are lacking. Indirect selection in spaced plants would be efficient, but spaced-plant traits need to be identified that correlate with seed yield in drilled plots. Spaced plants were studied of nine perennial ryegrass cultivars with a different seed production when grown in plots. Genotypic variation within cultivars for seed yield components and other traits was assessed in four environments in replicated clonal experiments. Extensive genotypic variation for most traits was present within each cultivar. Based on cultivar means of 25 or 50 genotypes, significant differences among cultivars were found for most traits. Cultivar-year interactions were found for most traits, but no significant cultivar-location interactions were generally found. Spaced-plant traits in general showed poor correlation to corresponding traits in drilled plots. Cultivars with contrasting seed yield in plots could not be distinguished on the basis of their spaced-plant traits and therefore no criteria for indirect selection for seed production in drilled plots could be identified in spaced plants.

Introduction

Seed yield in perennial ryegrass (*Lolium perenne* L.) is low. Selection for high seed yield should become a more important aspect of grass breeding (Elgersma, 1985). However, selection criteria for high seed production are lacking (Van Wijk & Duyvendak, 1984). In grass breeding, selection usually starts with large source populations based on widely-spaced plants. During commercial seed production grasses are sown in rows. Therefore, it is necessary to identify spaced-plant traits that correlate with the seed yielding capacity of a crop grown in drilled plots. In order to identify such traits an extensive study on variation for seed yield and its components was carried out both in drilled plots and in spaced plants. Significant and consistent differences for seed production in drilled plots were found among nine diploid perennial ryegrass cultivars (Elgersma, 1990a). The present study was designed to characterize these cultivars when grown as spaced plants.

Most reports concerning genetic variation for seed yield in grasses deal with either spaced plants (e.g., Burton & DeVane, 1953; Nguyen & Sleper, 1983; 1985; Elgersma et al., 1989; Rognli, 1987) or drilled plots (Evans & Muncey, 1977; Nordestgaard & Juel, 1979). However, Bean (1972) and Bugge (1987) studied both spaced plants and plots. Bugge (1987) found correlations between the performance of spaced plants of perennial ryegrass and their topcross progenies in drilled plots for ear length, ear number and thousand-grain weight, but not for seeds per spike or for seed yield. Unfortu-

nately, the sown crops were not fully developed due to a very low N-application.

The objectives of this paper are: (i) to estimate genetic parameters for seed yield components and other spaced-plants traits within cultivars; (ii) to assess the variation between cultivars, based on means of spaced-plant traits and (iii) to relate the performance of cultivars as spaced plants to their performance in drilled plots.

Material and methods

Plant material

Seeds of the late-flowering perennial ryegrass cultivars 'Semperweide', 'Wendy', 'Compas', 'Lamora', 'Perma', Barenza', 'Parcour', 'Trani' and 'Vigor' were obtained from the Government Institute for Research on Variaties of Cultivated Plants (RIVRO) and were sown in a greenhouse in April 1985. Fifty randomly chosen seedlings of each cultivar were transplanted to pots and grown outdoors during the summer. The 450 genotypes were cloned in June and further cloned in August into seven ramets. Four ramets of equal size were used in the present experiments, two were used in a competition experiment and one was kept as a spare.

Methods and experimental design

Experiments were conducted at two locations, 'Born' (sandy soil) and 'Nude' (river clay), these being 6 km apart in Wageningen, the Netherlands. The two soil types were chosen, because plant breeders usually observe spaced plants on sandy soil, whereas commercial seed production occurs mainly on clay soils. At each location two ramets of the 450 genotypes were planted in September. The plants were individually randomized, irrespective of cultivar, planted 0.75 m apart within two blocks, and each trial was surrounded by border plants. The size of both trials was 10.5 × 75 m. During the autumn and spring, weeds were hoed mechanically. Fertilizer was applied in March. More experimental details are described elsewhere (Elgersma, 1990a). Plants that died were replaced but these spare plants were excluded from the experiment. The plants were tied up to bamboo sticks when flowering to prevent tiller losses and to facilitate harvesting. Ten similar spikes were selected per plant and tied up separately. Harvest ripeness was visually assessed per individual plant according to the colour of the spikes and seeds and to the degree of seed shattering. Harvesting was carried out per plant with a sickle, the bundle of ten spikes being harvested separately. The spikes were cool-air dried, threshed by hand and the seed was cleaned before weighing.

In 1986/87 and in 1987/88 new experiments were carried out on sandy soil only. From the original 50 genotypes, 25 were taken at random per cultivar. The plant material was taken from the spare ramets that were grown in pots. In each of the new trials, two ramets were planted per genotype. In these experiments a randomized block design in two replications was used for the cultivars and the genotypes of a cultivar were randomized within the blocks.

The nine cultivars were grown simultaneously in drilled plots adjacent to the spaced-plant trials (Elgersma, 1990a; 1990b). The management of drilled plots was typical of that used in commercial seed multiplication (Meijer & Vreeke, 1988).

Observations

The spaced-plant traits assessed are shown in Table 1. Fertile tillers were only counted in 1986, because the experimental procedure was unsuitable.

Statistical analyses

Traits determined on tiller samples were averaged per plant. For both 1986 trials, separate analyses of variance of genotype means were carried out for each trait. The coefficient of genotypic variation (cv_g) cannot be presented for maturity dates, because it depends on the arbitrarily chosen initial date from which days are counted. In 1986 the

effect of blocks was not significant and therefore, in further analyses the blocks were ignored and complete randomization was assumed. The broad-sense heritability (h^2_b) on a genotype mean basis was calculated as $h^2_b = \sigma^2_g/(\sigma^2_g + \sigma^2_e/r)$ where r = 2. Expected gains from selection were calculated per trait on a two-clone mean basis, assuming a selection intensity of 10% (Burton & DeVane, 1953). Within each of the four trials phenotypic correlations were calculated between traits.

For cultivar comparisons, analyses of variance per trial were carried out on the cultivar means, averaged over spaced plants, for each trait (Weatherup, 1980). A model with nine cultivars in two replicates was used. Correlations were calculated between traits (based on cultivar means) within each trial. Cultivar means were also correlated between trials. For 1986, a combined analysis of variance of cultivar means was carried out to determine the effect of location and the cultivar-location interaction. Since in 1987 and 1988 only 25 genotypes were studied per cultivar, the results obtained in 1986 were analyzed based on these 25 genotypes as well, to enable a cultivar comparison across the four trials based on the same materials. A combined analysis of variance of cultivar means was carried out for the trials conducted on sandy soil to assess the effect of year and the year-cultivar interaction.

Cultivar means of spaced-plant traits were also correlated with seed yield, thousand-grain weight and other characteristics that has been assessed in crops grown in drilled plots (Elgersma, 1990a; 1990b).

Subsets of the 1986 data were made by selecting the 50% genotypes with the largest girth at ear emergence within each cultivar. Per location, cultivar means of spaced-plant traits were calculated and correlated with seed yield per plot (Elgersma, 1990a).

Table 1. Traits and their abbreviations. Traits 1–15 were determined on a plant basis and traits 16–20 on two representative tillers per plant, sampled three weeks after first anthesis. Traits 21–23 were calculated

1.	WIN	winterhardiness (visually rated in spring: 1 = poor, 5 = excellent) (only measured in 1986)
2.	GIS	plant girth (cm), measured in spring
3.	EE	ear emergence or heading date (days from April 1), when at least 3 ears are visible
4.	FLL	flag leaf width (mm) at ear emergence
5.	FLW	flag leaf length (mm) at ear emergence
6.	NPH	natural plant height (cm) at ear emergence
7.	GIE	plant girth (cm) at ear emergence
8.	FA	date of first anthesis (days from April 1)
9.	PA	date of peak anthesis (days from April 1)
10.	EA	date of the end of anthesis (days from April 1)
11.	PH	plant height (cm) when pulled up, after flowering
12.	HD	harvest date (days from April 1)
13.	FT	number of fertile tillers (only measured in 1986)
14.	SYP	seed yield per plant (g)
15.	SYS	seed yield per spike (g) from the selected spikes
16.	EL	ear length (mm) from the base of the lowest to the base of the upper spikelet
17.	IN	internode length (mm) from the upper internode to the base of the lowest spikelet
18.	SPK	number of spikelets per ear
19.	FL	number of florets in the central spikelet
20.	S	number of seeds in the central spikelet
21.	FSU	floret site utilization calculated per spikelet as (seeds/florets)*100%
22.	EEFA	days from ear emergence to first anthesis (heading period)
23.	FAHD	days from first anthesis to harvest (ripening period)

Results

Comparison of genotypes

Significant within-cultivar differences were found for most spaced-plant traits in each trial. The ranges within each cultivar were quite large, but no evident differences in range were found between the cultivars. Estimates of genetic parameters of each cultivar, obtained in 1986, are illustrated in Table 2 for seed yield per plant. Within each cultivar, significant differences were observed among the genotypes. The broad-sense heritabilities were rather high and did not differ significantly among the cultivars.

The ranges and genetic parameters for spaced-plant traits were rather similar for all cultivars. Therefore, the 1986 data were pooled for all the cultivars to present a general impression of the genetic variation for all traits. Various genetic parameters are shown in Table 3. The overall means at both locations were quite similar for the maturity dates, as well as for plant height, plant girth, numbers of spikelets, florets and seeds, floret site utilization and seed yield per spike. Mean values for other spaced-plant traits differed greatly between locations. In general, flag leaves and ears were longer on sand, whereas on clay the plants had longer upper internodes, more tillers and a much higher seed yield. The genetic parameters were quite similar at both locations, even for those traits that had different mean values. For example, although the seed yield per plant was almost twice as high on clay as on sand, at both locations it could be improved by 40% by selecting the top 10%. However, this could be an overestimation as it is calculated from broad-sense heritability. Genetic parameters differed among traits (Table 3). The heritabilities were very high for maturity dates. The heritabilities measured on clay were in general slightly higher than those measured on sand.

Correlations on a spaced-plant basis between seed yield per plant and other traits are presented in Table 4, and are more or less similar in the four trials. Although the correlation coefficients were rather low, most values were significant owing to the large numbers of plants. The most important spaced-plant traits related to seed yield per plant were plant girth at ear emergence, earliness, seed yield per spike and the number of fertile tillers (only measured in 1986).

Comparison of cultivars

In 1986, the nine cultivars differed significantly for all investigated traits at both locations, based on spaced-plant means. A combined analysis of variance showed significant effects of cultivar and location for most traits, but cultivar-location interac-

Table 2. Mean value ± Standard Error (SE), range, coefficient of genotypic variation (cv_g) and broad-sense heritability (h^2_b) of seed yield per plant in nine perennial ryegrass cultivars, measured at two locations in 1986 on a two-clone mean basis of 50 genotypes per cultivar. The cultivars are ranked according to their seed yield when grown in drilled plots

Cultivar	Sand				Clay			
	Mean ± SE	range	cv_g	h^2_b	Mean ± SE	range	cv_g	h^2_b
Trani	37* ± 2	1–67	25	0.41	71*** ± 3	34–138	29	0.78
Wendy	40*** ± 2	8–71	26	0.61	74*** ± 3	25–118	18	0.51
Semperweide	40*** ± 2	13–78	33	0.69	74*** ± 3	34–118	21	0.69
Vigor	38*** ± 3	3–77	38	0.68	69*** ± 3	10–132	27	0.75
Parcour	36*** ± 2	2–66	38	0.77	68*** ± 3	24–116	29	0.84
Perma	41*** ± 2	11–95	34	0.74	69*** ± 3	25–121	26	0.69
Compas	45*** ± 2	17–81	26	0.62	79*** ± 3	27–142	28	0.82
Barenza	37* ± 2	8–63	24	0.47	65*** ± 3	23–107	28	0.79
Lamora	38* ± 2	1–73	26	0.49	66*** ± 3	1–110	25	0.66

*, **, *** indicate the significance of differences between genotypes within each cultivar at $P \leq 0.05$, 0.01 and 0.001, respectively.

tions were generally not significant. Similar cultivar means were obtained using either 25 or 50 genotypes. The means were significantly correlated ($r \geq 0.80^{**}$) for almost all traits, indicating that 25 genotypes (based on two plants per genotype) are sufficient to provide a good estimate of the cultivar mean for most traits. The mean performance of the cultivars in four trials, based on 25 genotypes, is presented in Table 5. Significant differences among cultivars were found for most traits. The ranking of cultivar means differed among trials. For most traits the correlation of cultivar means between locations was significant, but there was no significant correlation between years. Cultivar-year interactions were therefore more important than cultivar-location interactions.

Correlations on a cultivar mean basis between seed yield per plant and other traits were generally of the same magnitude in the four trials (data not shown). Seed yield per plant was positively correlated with earliness, but this was only significant in 1986. In all trials, a negative correlation (ranging from $r = -0.72^{*}$ to $r = -0.83^{**}$) was found between seed yield per plant and the number of spikelets per ear. No other traits showed significant correlations with seed yield per plant.

Table 3. Mean value, coefficient of genotypic variation (cv_g) and broad-sense heritability (h^2_b) of 14 spaced-plant traits of perennial ryegrass, measured at two locations in 1986 on a two-plant mean basis of 450 genotypes pooled over nine cultivars. For abbreviations of traits see Table 1

Trait	Sand			Clay		
	Mean	cv_g	h^2_b	Mean	cv_g	h^2_b
WIN	3.9	15	0.58	4.4	13	0.66
EE	72	–	0.95	71	–	0.96
GIE	132	10	0.61	131	9	0.66
FLL	246	12	0.64	208	14	0.69
PH	71	8	0.61	74	9	0.71
FT	344	17	0.46	485	16	0.49
SYP	39	31	0.63	71	27	0.75
SYS	0.18	25	0.62	0.20	24	0.65
EL	246	14	0.73	237	13	0.76
IN	167	16	0.54	220	12	0.71
SPK	24.2	12	0.71	24.9	12	0.83
FL	9.8	14	0.69	10.0	12	0.68
S	7.5	24	0.72	7.4	21	0.66
FSU	75	19	0.51	72	16	0.63

Spaced plants versus drilled plots

The cultivars 'Trani' and 'Wendy' consistently outyielded 'Lamora' and 'Barenza' when grown in drilled plots (Elgersma, 1990a). However, these cultivars cannot be separated on average values of any single spaced-plant trait (Table 5). Most spaced-plant traits were poorly correlated with seed yield per plot (Table 6). Figures 1 and 2 show the relationship of seed yield per plant and seed yield per spike, respectively, with total seed yield per plot. Seed yield per plant was low in the most productive cv. 'Trani', whereas the average yielding cv. 'Compas' had the highest seed yield per plant (Fig. 1). The relationship between plot yield and seed yield per spike (Fig. 2) was better than that between plot yield and seed yield per plant (Fig. 1).

A significant correlation was found between seed yield per plot and winter damage in spaced plants in 1986, but this is not a suitable selection criterion. In all trials, a small plant girth at ear

Table 4. Phenotypic correlations between seed yield per plant and six other spaced-plant traits of perennial ryegrass, in four trials. For abbreviations of traits see Table 1

	Trial			
	'86 clay[1]	'86 sand[1]	'87 sand[2]	'88 sand[2]
EE	-0.22^{**}	-0.17^{**}	-0.40^{**}	-0.40^{**}
GIE	0.36^{**}	0.34^{**}	0.39^{**}	0.22^{**}
SYS	0.31^{**}	0.40^{**}	0.42^{**}	0.38^{**}
EL	0.01	0.08	−0.08	0.11^{*}
PH	0.22^{**}	0.29^{**}	0.22^{**}	0.16^{**}
SPK	−0.05	-0.13^{**}	-0.25^{**}	-0.16^{**}
FL	0.19^{**}	0.19^{**}	0.17^{**}	0.17^{**}
S	0.27^{**}	0.28^{**}	0.25^{**}	0.25^{**}
FSU	0.21^{**}	0.20^{**}	0.17^{**}	0.13^{*}
FT	0.38^{**}	0.48^{**}	–	–
EEFA	0.14^{**}	0.28^{**}	0.33^{**}	0.19^{**}
FAHD	0.04	0.09^{*}	−0.05	0.21^{**}

*, ** indicates a significance at $P \leq 0.05$ and 0.01, respectively.
[1] based on 900 plants.
[2] based on 450 plants.

emergence showed the best correlation with high seed yield per plot (Table 6). To avoid any possible, unwanted influence of weak plants, subsets of the 50% genotypes with the largest girth at ear emergence (an indication of vigor) within each cultivar were analyzed using the 1986 data. This resulted in higher cultivar means for girth, seed yield per plant, ear length and fertile tiller number at both locations (data not shown). The cultivar ranking for girth did not change substantially after elimination of the smallest plants (again 'Barenza' had the largest girth), unlike the ranking for seed yield per plant ('Trani' and 'Vigor' had a relatively higher mean seed yield per plant). Correlations be-

Table 5. Means of various traits of nine perennial ryegrass cultivars and LSD-values (5%) between cultivars, calculated from analyses of variance based on cultivar means, within four trials. Per trial, the minimum is underlined and the maximum in *italics*. For abbreviations of cultivar names see text. For abbreviations of traits see Table 1

Trait	Trial	Cultivar									Mean	LSD (5%)
		S	W	C	L	Pe	B	Pa	T	V		
EE	'86 clay	71	68	68	*75*	69	*75*	71	74	73	**71*** **	1
	'86 sand	71	69	68	*76*	69	75	72	74	74	**72*** **	1
	'87 sand	76	72	69	*79*	72	*79*	74	77	79	**75*** **	3
	'88 sand	61	58	58	*64*	58	*64*	61	63	63	**61*** **	3
GIE	'86 clay	129	130	*137*	132	131	*137*	124	125	124	**131*** **	4
	'86 sand	132	126	137	138	132	*141*	125	123	127	**132*** **	5
	'87 sand	100	95	113	106	105	*117*	100	103	103	**105*** **	9
	'88 sand	93	98	97	96	94	*110*	95	94	106	**98** **	7
SYP	'86 clay	76	73	*83*	63	71	60	60	64	67	**69** **	6
	'86 sand	42	39	*47*	37	40	37	33	35	37	**38***	5
	'87 sand	20	18	*27*	16	13	17	19	13	17	**18*** **	6
	'88 sand	22	*24*	19	16	16	16	14	14	21	**18** **	5
SYS	'86 clay	*0.22*	0.21	0.19	0.19	0.20	0.18	0.17	0.20	0.20	**0.20*** **	0.01
	'86 sand	0.19	0.19	0.18	0.18	0.19	0.16	0.16	*0.20*	0.18	**0.18**(*)**	0.02
	'87 sand	*0.12*	*0.12*	*0.12*	0.08	0.08	0.07	0.10	0.10	0.10	**0.10*** **	0.00
	'88 sand	*0.03*	*0.03*	*0.03*	0.02	0.02	0.01	0.02	0.02	0.02	**0.02*** **	0.01
SPK	'86 clay	25	24	24	*26*	25	*26*	25	*26*	25	**25.1*** **	0.7
	'86 sand	24	23	23	25	24	*26*	25	25	25	**24.4*** **	0.8
	'87 sand	22	22	22	23	*24*	*24*	23	*24*	24	**23.1*** **	1.2
	'88 sand	25	25	26	27	27	*28*	26	27	26	**26.3*** **	1.3
S	'86 clay	7.2	7.9	*8.2*	7.1	7.4	6.9	6.5	7.3	6.2	**7.2** **	0.6
	'86 sand	7.5	*8.4*	7.9	7.2	6.7	7.5	6.2	7.5	6.6	**7.3***	0.6
	'87 sand	6.8	6.9	*7.5*	6.0	6.0	6.7	5.8	6.8	6.1	**6.5*** **	0.8
	'88 sand	4.3	5.5	*5.6*	4.5	5.4	4.7	4.6	5.1	3.8	**4.8** **	0.6
FSU	'86 clay	0.71	0.74	*0.77*	0.72	0.69	0.71	0.67	0.74	0.66	**0.71**(*)**	0.05
	'86 sand	0.76	0.79	*0.77*	0.73	0.70	*0.80*	0.66	0.76	0.74	**0.74***	0.04
	'87 sand	0.73	0.71	*0.78*	0.64	0.67	0.73	0.67	0.70	0.72	**0.71** **	0.07
	'88 sand	0.58	0.68	*0.71*	0.59	0.67	0.64	0.59	0.67	0.54	**0.62*** **	0.06
EEFA	'86 clay	14	*16*	15	12	*16*	13	14	13	13	**14*** **	1
	'86 sand	14	*16*	*16*	12	*16*	13	14	13	12	**14*** **	1
	'87 sand	20	22	*24*	18	23	20	22	21	18	**21*** **	2
	'88 sand	19	*21*	19	18	20	19	19	19	20	**19*** **	1

***, **, *, (*) indicates a significance at $P \leq 0.001$, 0.01, 0.05 and 0.10, respectively.

tween spaced-plant trait means (based on the largest plants) and seed yield per plot were not affected (compared to the data presented in Table 6).

Spaced-plant trait means (original data) were also correlated with thousand-grain weight assessed in drilled plots (Elgersma, 1990a). In contrast to cultivars with a different seed yield (Table 6), cultivars with a different thousand-grain weight could be distinguished based on various spaced-plant traits. Cultivars that produce heavy seeds when grown in plots tended to have spaced plants with a small girth at ear emergence, long flag leaves, long ears with few spikelets and a high seed yield per spike in all trials.

Various spaced-plant traits were also correlated with corresponding traits assessed in drilled plots that had been determined in some trials (Elgersma, 1990b). Most traits showed no significant correlations, e.g., there was no correlation between the average number of ears per spaced plant and the number ears per m^2 in 1986 at either location. The number of seeds per spikelet, the number of florets per spikelet and floret site utilization in spaced plants were not correlated with the same traits assessed in crops grown in 1987 and 1988. However, there was a significant correlation between ear length in spaced plants and in plots in 1988 ($r = 0.82^{**}$) and also between the number of spikelets per ear in spaced plants and plots in 1987 ($r = 0.90^{**}$), but not in 1988 ($r = 0.64$).

Table 6. Correlations between cultivar means of various spaced-plant traits and seed yield per plot within each of four trials, and the correlations between overall means of spaced-plant traits and seed yield per plot, averaged over the four trials. For abbreviations of traits see Table 1

	Trials				Mean
	'86 clay	'86 sand	'87 sand	'88 sand	
WIN	−0.66(*)	−0.89**	–	–	–
EE	−0.28	−0.30	−0.31	−0.47	−0.38
GIE	−0.20	−0.57	−0.67*	−0.51	−0.65(*)
FT	−0.21	−0.28	–	–	–
SYP	0.54	0.27	0.04	0.17	0.22
SYS	0.23	0.48	0.72*	0.41	0.52
EL	0.20	0.33	0.40	0.16	0.42
SPK	−0.54	−0.41	−0.23	−0.52	−0.47
FL	0.33	0.37	0.03	0.14	0.23
S	0.30	0.42	0.19	−0.10	0.27
FSU	0.18	0.39	0.29	0.14	0.17
EEFA	0.30	0.31	0.27	0.50	0.45
FAHD	0.13	0.38	−0.34	0.03	−0.19

**, *, (*) indicates a significance at $P \leq 0.01$, 0.05 and 0.10, respectively.

Table 7 shows overall means of the most relevant traits determined in spaced plants and plots. The environment has an obvious effect on various traits e.g., maturity and the length of developmental periods are mainly affected by the year, whereas yield for both spaced plants and plots is also greatly affected by location. The yield difference between locations in 1986 may be due to drought stress on sand. Seed yields in 1987 and 1988 were similar in spaced plants, but the yields in plots were much lower in 1988 than in 1987, possibly due to higher losses in the heavily lodged crop during the wet summer of 1988. Although spaced plants had a similar seed yield in 1987 and 1988, seed yield per spike was much lower in 1988 than in 1987. In 1988, spikes contained more spikelets but fewer seeds per spikelet and the floret site utilization was lower. Seeds were also smaller and lighter in 1988, both in spaced plants and plots.

Discussion

Among the nine cultivars small, significant differences occurred for the traits investigated in this study, while within each cultivar large and highly significant differences occurred among genotypes. This agrees with similar observations in perennial ryegrass (Bugge, 1987), Westerwold ryegrass (Elgersma et al., 1989) and tall fescue (Griffiths et al., 1966; Burton & DeVane, 1953). It has therefore often been suggested that selection within cultivars could be effective for high seed yield. From this study too, it is clear that selection perspectives are present for important spaced-plant traits, including seed yield per plant. However, this is only useful if high seed yielding genotypes, when combined into a synthetic variety, produce offspring with a high seed yield when grown in drilled plots.

This study reveals, that most spaced-plant traits showed no significant correlation with seed yield

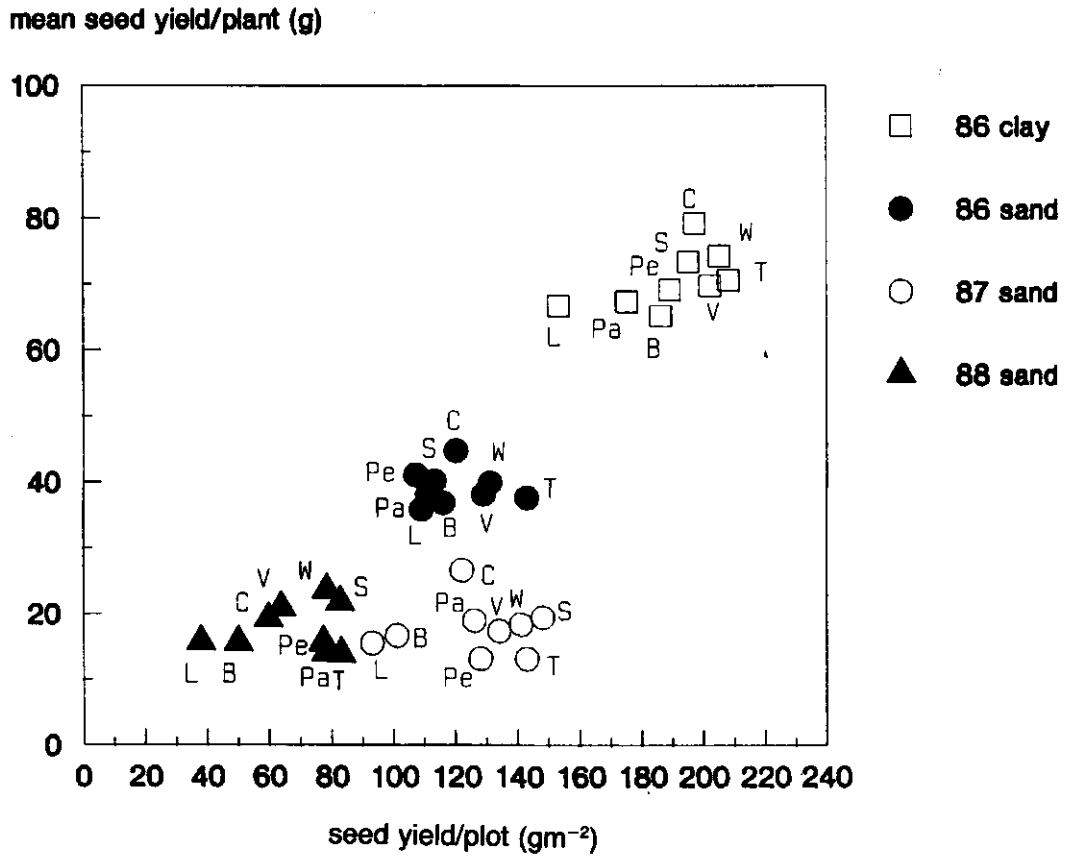

Fig. 1. The relationship between the mean seed yield per spaced plant and the total seed yield per plot of nine perennial ryegrass cultivars in four trials. Cultivar names are abbreviated with their first letter(s), for names see text.

per plot. The negative correlation between plant girth and seed yield per plot was mainly caused by the very large average plant girth in the low yielding cultivar 'Barenza'. Therefore, in spaced plants no useful selection criteria were found for seed yielding capacity in drilled plots. The correlation between performance as single plants and in swards is high for traits such as ear emergence (Rognli, 1987; Cooper, 1959), but low for forage yield (e.g., Hayward, 1983). This discrepancy is believed to be caused mainly by increased competition in swards. Therefore, Burton & DeVane (1953) and Rognli (1987) expect a fairly high correlation between seed yield of spaced plants and seed yield in plots, as the stand in a drilled plot is less dense than in a sward. Indeed, a high correlation between the seed yield of spaced plants and sown plots was reported by Knowles (1977) in *Agropyron intermedium* and Bean (1972) in tall fescue and timothy. However, Bugge (1987) found in perennial ryegrass no correlation between seed yield of spaced plants and that of their progenies sown in rows. Similarly in this study, no consistent correlation between seed yield of spaced plants and seed yield in plots was found. This may be due to plant spacing in general: the more heterogeneous tillering in spaced plants compared to drills may be caused by the relatively high light penetration in the former. Late-emerging tillers survive under such conditions, while the mortality of such tillers in plots is high due to competi-

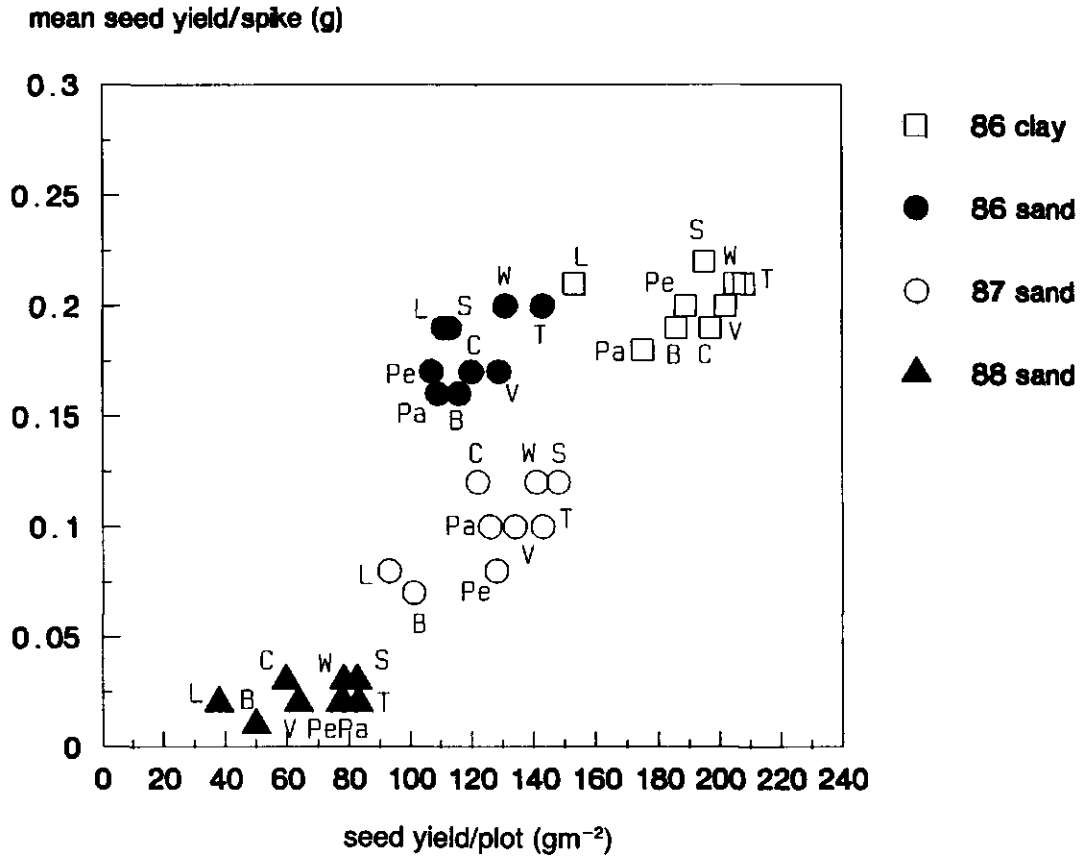

Fig. 2. The relationship between the mean seed yield per spike, averaged over spaced plants, and the total seed yield per plot of nine perennial ryegrass cultivars in four trials. Cultivar names are abbreviated with their first letter(s), for names see text.

tion of the earlier-emerged tillers. Thus heterogeneity in spaced plants is larger than in plots. The plants were also tied up after flowering which reduced tiller losses compared to those in lodged plots.

For these reasons it was expected that spaced-plant traits that depend less on plant spacing, such as seed yield per spike, numbers of spikelets, florets and seeds, floret site utilization and ear length, relate better to corresponding plot traits than spaced-plant traits such as seed yield per plant or tiller number. However, except for ear length (which was also found by Bugge (1987)) and possibly spikelet number, this was not the case. Moreover, ear length and yield components determined in plots were not related to seed yield per plot (Elgersma, 1990b). The ranking of cultivar mean values of most spaced-plant traits differed among years (this study), whereas in plots the ranking for seed yield was similar for different environments (Elgersma, 1990a). In general, the relative performance of populations in spaced-plant trials is greatly affected by environmental differences (Davies & Snaydon, 1989). Nguyen & Sleper suggested in 1983 that spaced-plant selection for earliness would improve seed yield in tall fescue, but in 1985 they recommended family selection rather than individual plant selection to reduce environmental effects that occur in spaced plants. In orchardgrass Stratton & Ohm (1989) also found large genotype-

location interactions and therefore recommended family selection rather than spaced-plant selection to improve seed yield. However, Bugge (1987) argued that in perennial ryegrass family selection was less succesful than single plant selection.

Another reason for the discrepancy between spaced-plant and plot performance may be the composition of the plant sample of a cultivar. The sample size used here seems sufficiently large to characterize the cultivar mean, as mean values calculated from either 25 or 50 genotypes were similar and showed a high degree of correlation. However, the spaced plants were taken at random and grown without competition; consequently weak genotypes were not eliminated, as happens in drilled plots. Extremely late maturing genotypes are also eliminated in plots, since they are overgrown. Therefore, the spaced-plant samples probably contain a larger range in vigour and maturity than the plots. However, the correlation between seed yield per plant and seed yield per plot did not improve when only the largest plants were included. Finally, the poor relationship between spaced plants and plots might be due to the rather loose genetic relationship between plants of a synthetic variety. The relationship between spaced plants and their progenies sown in plots would be more precise than that between random plant samples and sown cultivars.

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Table 7. Mean values of spaced-plant traits and of traits determined in crops grown in drilled plots of perennial ryegrass in four environments (data in part from Elgersma 1990a; 1990b). For abbreviations of traits see Table 1

	Trait											
	EE	FA	PA	HD	EEFA	FAHD	EL	SPK	F	S	FSU	SYP
Spaced plants												
Trial												
'86 clay	71	86	87	112	14	26	237	25	10	7.4	72	71
'86 sand	72	86	87	115	14	27	246	24	10	7.5	75	39
'87 sand	75	96	99	125	21	29	252	23	9	6.5	71	18
'88 sand	61	80	85	114	19	34	216	26	8	4.8	62	18
	EE	FA	PA	HD	EEFA	FAHD	EL	SPK	F	S	FSU	SEED YIELD (gm^{-2})
Drilled plots												
Trial												
'86 clay	75	86	92	122	12	36	–	–	–	–	–	206
'86 sand	72	87	94	120	15	33	–	–	–	–	–	133
'87 sand	73	93	95	129	20	36	–	20	–	4.8	83	126
'88 sand	60	73	81	114	13	41	175	23	5	3.6	73	68

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GENERAL DISCUSSION

Floret site utilization

The reproductive potential of a grass seed crop is determined by the number of ears per unit area, the number of spikelets per ear and the number of florets per spikelet. The degree to which this potential is realized in terms of yield depends on the proportion of florets that produce seed (biological floret site utilization) and the size of the individual seed. Many florets are unproductive. The large difference between potential and actual seed production is due to the poor capacity of florets to mature seeds (Marshall, 1985). Losses occur during the developmental processes occurring from anthesis onwards, namely pollination, fertilization, seed set and seed development (Hill, 1980). Furthermore, in an economical sense floret site utilization is also strongly decreased by harvesting and cleaning losses (Chapter 1).
The reasons for unproductive florets can be many and can only be resolved by detailed observations on the development and fate of individual florets with time from the emergence of the inflorescence to its maturation (Marshall, 1985). For practical breeding, however, screening methods should be simple as large numbers of plants have to be observed.

Pollination and fertilization

Minimum temperature at anthesis and during the week after anthesis was the main environmental factor related to seed yield in field experiments (Hampton and Hebblethwaite, 1983), but the precise effect of low temperature is not clear (Hebblethwaite, 1985). Our studies under controlled conditions at four temperatures revealed, that both the genotype of the mother and temperature affected pollen performance (Chapter 2). In excised ovaries, (semi-*in vitro*) the genotype of the mother affected pollen tube growth and there was also a significant mother-temperature interaction. Selection for tolerance to low temperatures at anthesis seems promising. At present, various *in vitro* systems are being compared with the *in vivo* situation in order to develop screening methods for studying fertilization and floret site utilization.

Seed development

When a floret is not pollinated or the egg cell is not fertilized, the ovule and ovary degenerate (Chapter 3). In normal seed development, three stages of seed development can be distinguished (Hyde *et al.*, 1959): the growth stage (10 days), the food reserve accumulation stage (10-14 days) and the ripening stage (3-7 days). We found that the large majority of the unproductive florets showed ovule degeneration within a few days after flowering. In general, seeds are most susceptible to abortion during the growth stage (Evenari, 1984). We rarely observed seed abortion in later stages, which agrees with findings of Meijer (1985).

Within an individual spikelet Anslow (1963; 1964) and Burbidge *et al.* (1978) found a marked decline in the capacity of florets to set seed from the basal to the distal florets. These authors also reported that seeds of distal florets were more

susceptible to abortion. This pattern suggests competition for assimilates or mineral nutrients within the spikelet, with the basal florets developing at the expense of distal florets (Marshall, 1985). Based on these ideas, workers at the University of Nottingham suggested that the gap between potential and realized seed yield is caused by low floret site utilization, due to assimilate shortages. These are thought to be caused by lodging and by competition from the elongating tiller and from vetetative tillers (Hampton and Hebblethwaite, 1984; 1985). Hebblethwaite (1985) therefore advocated spraying different chemicals to prevent lodging, to increase transfer of assimilates to the seed and to prevent shattering in an upright crop. However, the present trend is to reduce chemical treatments. Moreover, our data on spaced plants (Chapter 3) and drilled plots (Chapter 6) do not sustain the above theory. Empty florets were found more frequently in the higher positions of a spikelet, but were also quite often found in all other floret positions. No clear pattern in the position of unproductive florets was found. This was recently confirmed by observations in a sown crop (Marshall and Ludlam, 1989), where there was no position effect and there was neither an effect of erect spikes (more assimilation) nor of removal of other spikelets (less competition) on floret site utilization or on seed growth. This indicates that pollination is not hampered in a lodged crop and that the majority of the distal seeds within a spikelet do not abort. Therefore, genetic or cytological factors were probably responsible for the failure of seed development, rather than physiological stress due to competition between sinks.

Well-developed seeds started to shatter from 3.5 weeks after first anthesis.
Seed shattering is an important source of yield loss (Stoddart, 1964). Shattering resistance (seed retention) does not affect forage quality and seems a very desirable trait, provided that the recovery of seeds during threshing is not impaired. In our study of the cytological basis of this trait, genotypes with different seed retention did not show differences in abscission layer development and seed shattering mechanism (Chapter 4). Just below each floret an abscission layer was detected, which was already present at ear emergence. During anthesis and ripening, the number of cell layers of the abscission layer in the central area increased slightly. No degradation of the abscission layer cells was detected, indicating that abscission took place mechanically.

Field experiments
Preliminary studies with Westerwold ryegrass (*Lolium multiflorum* var. *westerwoldicum*) (Elgersma *et al.*, 1989), perennial ryegrass and red fescue (*Festuca rubra*) highlighted some of the restrictions of the main field experiment (Chapters 5 to 8). As the variation among genotypes within a cultivar proved to be large, a sufficiently large number of randomly taken spaced plants must be taken to determine within cultivar variation. Cloning the plants provides a means to estimate genotypic and environmental variation due to year, location and plant age. It was also concluded, that the advantages of cloning the plants seem to be larger than the disadvantage (possible introduction of within-genotypic variation as a result of different ramet size). This view was recently confirmed by Davies and Snaydon

(1989): even a ten-fold difference in initial ramet size had little effect on the subsequent performance of contrasting genotypes of the grass *Anthoxanthum odoratum*.
In practical breeding, spaced plants are often selected in breeders nurseries on sandy soil, whereas seed multiplication occurs mainly on clay in the Netherlands. Therefore the experiments were conducted on a sandy and a clay soil. Agronomic management was carried out according to that in practice in the Netherlands, but seed was harvested with a fodder harvester to minimize harvest losses in order to get the most reliable seed yield data.

Generative versus vegetative traits
Already in 1966 Lewis pointed out, that the herbage breeder has always to consider the consumer advantage of a cultivar in relation to the cost of the seed mixture. The breeder is challenged to combine vegetative qualities and high stable seed yield in one cultivar. Those two characteristics are believed to act adversely on each other (van Wijk, 1985). According to Bugge (1987), late-flowering cultivars have a poor seed yield. Breese and Hayward (1972) cautioned against the inclusion of progeny testing in the breeding scheme for grasses, as this would give too much importance to sexual reproduction, when traits associated with asexual reproduction have to be improved! Therefore, Bean (1972) stated that owing to the negative feedback on forage production, it is not desirable to increase the size of the reproductive system. Instead, its efficiency should be increased which can be achieved by improving floret site utilization.
The 450 genotypes that were studied for seed yield components were also grown in a timothy sward to assess their competitive ability, which is correlated with persistence, an important vegetative trait (van Dijk and Winkelhorst, 1978). Observations from 1985 to 1988 (Elgersma and de Vos, unpublished) showed that competitive ability differed largely among the genotypes and was not correlated with any other spaced-plant trait. Therefore, no evidence was found for a negative correlation between generative and vegetative traits.
Nine existing cultivars were used to ensure a high agronomic quality. Some cultivars were known to differ for seed yield in practice, although the differences are not extremely large as the very low yielding populations are not maintained in practice.

Drilled plots
Significant, reproducable differences for seed yield were found among the cultivars in twelve trials (Chapter 5). The highest yielding cultivar was superior over a wide range of environments and the seed yield of the poorest cultivar was on average only 64 % of that of the best cultivar.
This implies that vegetative quality and high stable seed yield can be combined in one cultivar. An interesting question is how high stable seed yield is realized in a crop. Which yield components are most important for high seed yield, are they suitable for indirect selection and how can a breeder select for high stable seed yield?
Significant cultivar differences also occurred for thousand-grain weight, but the ranking of the cultivars for seed yield and for thousand-grain weight were different.

Therefore, thousand-grain weight cannot be used for indirect selection for seed yield. The calculated number of seeds per unit area was strongly correlated with seed yield. The number of seeds is determined by seed yield components such as fertile tiller number, numbers of spikelets per ear and seeds per spikelet, and floret site utilization. However, these components were very variable within plots and often cultivar differences were not significant within a trial and not consistent in other trials. Cultivar differences for seed yield could neither be predicted from seed yield components assessed in drilled plots nor from crop physiological traits (Chapter 6). The discrepancy between the number of seeds determined in samples prior to harvest and the calculated number of seeds after harvesting, threshing and cleaning is large. This may be a reason for the poor correlation between yield components and seed yield, because differences in seed yield components may be overruled by losses during harvesting and cleaning. From experiments with different harvesting and threshing techniques (Elgersma and van Hateren, unpublished) it was concluded, that cleaning losses are probably responsible for the majority of the "lost" seeds. These seeds may be in part immature (too small or too light) or may become damaged, so that in samples they were scored as a seed but afterwards they are cleaned out. Further study of the distribution of seed size and seed weight within cultivars is needed.

The results presented in Chapters 5 and 6 reveal, that it is best to determine seed yield per plot, irrespective of how the components contribute to the resulting yield. When a breeder wants to assess the seed yielding capacity of a population in plots, border effects should be minimized and each plot should be harvested individually according to its harvest ripeness. Since no indirect traits were identified, seed yield itself must be determined. In a seed yield trial with various cultivars a pollen mixture is present in contrast to commercial multiplication, where only pollen from the cultivar itself is present. When new populations with insufficient compatibility are tested, this might result in overestimation of the seed production. However, in the present study with the existing cultivars this was not the case.

Spaced plants

Indirect selection for seed yield per plot in spaced plants would be very useful. The genotypic variation within each cultivar between spaced plants was large. Earliness, ear length, the number of spikelets per ear and flag leaf width had a high narrow-sense heritability in spaced plants and therefore show promise for selection (Chapter 7). For succesful indirect selection for seed yield, spaced-plant traits should have a high correlation with seed yield per plot in addition to a high heritability. Therefore, correlations were calculated between cultivar means of spaced-plant traits and seed yield per plot of the nine cultivars (Chapter 8). The correlations were generally low, for example the two highest yielding cultivars were dissimilar for most traits and the lowest yielding cultivar could not be distinguished from most other cultivars. A small plant girth at ear emergence and a high seed yield per spike showed the best correlation with high seed yield per plot, but unfortunately the narrow-sense heritabilities of these traits were very low. The traits with a high heritability were not correlated with seed yield per plot. Moreover, within each cultivar individual

genotypes showed large differences for all traits. Therefore, no useful selection criteria for seed yield per plot could be identified in spaced plants.

Implications for breeding

There are cultivar differences for seed yielding capacity in drilled plots. High and low yielding cultivars could not be distinguished based on their growth rythm, dry matter accumulation and partitioning, thousand-grain weight, fertile tiller number or other crop physiological traits. No independent characters were identified in crops grown in drilled plots that correlate with total seed yield per plot.
High and low yielding cultivars could neither be distinguished based on their spaced plants. Spaced-plant selection does not seem promising for indirect selection for high seed yield in drilled plots, because no spaced-plant traits were identified with a high heritability and a high correlation with seed yield in drilled plots. However, the heritability was based on spaced plants and these are more variable than seed crops in plots; therefore the relation between seed yield of half-sib families sown in drilled plots and spaced-plant traits of their mothers is currently being investigated.

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SAMENVATTING

Genetische, cytologische en fysiologische aspekten van zaadopbrengst in Engels raaigras (*Lolium perenne* L.)

Probleemstelling

Engels raaigras is een belangrijke grassoort, maar in de commerciële graszaadteelt zijn de zaadopbrengsten laag en wisselvallig. Dit heeft meteorologische, bodemkundige, teeltkundige en genetische oorzaken. Het proefschrift richt zich op dit laatste aspekt.

Grassenkwekers hebben zich in het verleden geconcentreerd op de verbetering van vegetatieve eigenschappen en hebben rassen geproduceerd voor o.a. weilanden, sportvelden en gazons. Daarbij kwamen de zaadproduktie eigenschappen pas op de tweede plaats. De zaadopbrengsten van grassen namen in het verleden toch wel toe door verbetering van de teelttechniek. Bovendien vreesden de kwekers dat selektie op zaadopbrengst de gebruikswaarde zou aantasten, omdat er een tegenstelling lijkt te bestaan tussen gebruikswaarde (vegetatieve kenmerken) en zaadopbrengst (generatieve kenmerken) van grassen. Nu het aantal kwalitatief goede grasrassen door veredeling sterk is toegenomen en het verschil in landbouwkundige waarde tussen de rassen veel kleiner is geworden, wordt het commerciële succes van een nieuw ras steeds meer bepaald door de produktiekosten van het graszaad. Momenteel zijn de perspektieven voor verdere verhoging van de zaadopbrengst door betere teelttechniek afgenomen. Kwekers krijgen daarom steeds meer belangstelling voor genetische verschillen in zaadproduktie. Het is daarbij nodig om díe componenten van zaadopbrengst te verbeteren, die de gebruikswaarde niet aantasten. Het voornaamste doel in de grassenveredeling is dus het combineren van goede vegetatieve èn generatieve prestaties binnen één ras. Een kweker begint doorgaans met aparte planten en wil zo snel mogelijk selekteren. Het is echter de vraag in hoeverre selektie voor zaadproduktie in een gezaaid veld zinvol is op basis van kenmerken in aparte planten. Doelstelling van dit onderzoek is het opsporen van selektiecriteria voor zaadopbrengst in Engels raaigras.

Inleiding

Uit inleidende proeven werd geconcludeerd, dat de relatie veld - aparte plant onderzoek behoefde, dat genetisch onderzoek naar zaadopbrengst gekoppeld moest worden aan gewasfysiologisch onderzoek en dat anatomisch/cytologisch onderzoek naar bestuiving, bevruchting, zaadontwikkeling, abortie en zaaduitval nodig was. Er is dus gekozen voor een multidisciplinaire aanpak van het probleem.

In hoofstuk 1 wordt de graszaadopbrengst omschreven als het produkt van de volgende opbrengstcomponenten:
-aantal halmen per m^2
-aantal bloempakjes per halm
-aantal bloemen per bloempakje
-bloembenutting
-gemiddeld zaadgewicht.

Uit de literatuur blijkt, dat vooral bloembenutting (het percentage bloemen dat zaad oplevert) een belangrijke opbrengstcomponent is. Daarnaast zouden ook faktoren als zaaduitval, spreiding in rijptijd en legering erg belangrijk zijn voor de zaadopbrengst. Bloembenutting kan worden opgevat in biologische zin maar ook in economische zin; hierbij wordt uitgegaan van het aantal zaden dat na oogsten, dorsen en schonen overblijft. Bloembenutting wordt bepaald door een aantal processen, zoals bestuiving, bevruchting, zaadontwikkeling en zaaduitval.
Fundamentele kennis over genetische en fysiologische aspekten van deze processen ontbreekt. Deze processen zijn daarom in detail bestudeerd.

Cytologisch onderzoek
Tijdens de bestuiving landt pollen op de stempel, waarna de pollenkorrels kunnen kiemen en de pollenbuizen ingroeien. Pollenbuisgroei in een stempel kan met een UV-microscoop worden waargenomen. In een oriënterend onderzoek werden genetische verschillen voor snelheid van pollenbuisgroei bij verschillende temperaturen gevonden. In hoofdstuk 2 wordt onderzoek beschreven naar de snelheid van pollenbuisgroei in genotypen met een verschillende zaadopbrengst, bij verschillende temperaturen. Er is zowel met intakte planten gewerkt (*in vivo*) als met vruchtbeginsels op agar in een petrischaal (semi-*in vitro*). Vooral het genotype van de moederplant en de temperatuur waren van belang voor de snelheid van pollenbuisgroei in de stempel.

Na bestuiving kan bevruchting optreden en vervolgens kan zich een zaad ontwikkelen. Zaadzetting en zaadvulling zijn met een binoculair en ook door middel van coupes onderzocht in aren, afkomstig van aparte planten (hoofdstuk 3). Degeneratie van vruchtbeginsels trad vooral op binnen enkele dagen na de bloei. Abortie van zaden in latere ontwikkelingsstadia kwam weinig voor. Niet-gevulde zaden kwamen aan de uiteinden van de bloempakjes iets vaker voor, maar werden ook op alle andere posities binnen de aar gevonden. Daarom is competitie tussen zich ontwikkelende zaden waarschijnlijk niet de reden voor degeneratie, maar zijn hiervoor wellicht genetische en cytologische faktoren verantwoordelijk.

Uitval van zaden is een verliespost in de graszaadteelt. Hierdoor treedt niet alleen opbrengstderving op, maar kan ook een opslagprobleem ontstaan. Tijdens de evolutie en domesticatie van de granen is de zaaduitval verminderd, doordat mutaties optraden in de breuklaag tussen het zaad en de stengel, de abscissielaag. Uit waarnemingen aan aparte planten in het veld, waarbij halmen ofwel bij rijpheid of één of twee weken daarna werden geoogst, bleek dat sommige genotypen in overrijpe staat nog een hoge zaadopbrengst hadden terwijl andere veel zaad verloren hadden. Halmen van vier vastzadige en drie loszadige genotypen werden individueel gemerkt en met intervallen geoogst vanaf schieten tot rijpheid. De halmen werden gefixeerd en met een ultra-microtoom werden coupes gesneden van de abscissielaag. De abscissielaag was tijdens het schieten al aanwezig en tijdens bloei en zaadvulling werden meer cellagen gevormd in het centrale deel. Abscissie gebeurde niet biochemisch, door het oplossen van celwanden, maar mechanisch, door scheuring. Er konden geen duidelijke anatomische verschillen tussen vastzadige

en loszadige genotypen worden aangetoond.

Veldproeven

Het veldonderzoek richtte zich op de genetische achtergronden van verschillen in zaadproduktie. Uit vooronderzoek bleek dat de variatie tussen planten uit een ras erg groot is, zodat een behoorlijke steekproef moet worden genomen. Door individuele zaailingen te scheuren (kloneren) kunnen de jaar-, locatie- en plantleeftijd-effekten worden ontstrengeld en kunnen de genetische en milieuvariatie worden geschat. De voordelen van klonen lijken ruimschoots op te wegen tegen de nadelen (mogelijke introduktie van extra binnen-kloon-variatie als gevolg van verschillen in plantgrootte).

Negen diploide rassen van het weidetype van Engels raaigras zijn vergeleken, nl. Semperweide, Wendy, Compas, Lamora, Perma, Barenza, Parcour, Trani en Vigor. De rassen verschillen weinig in doorschietdatum. Dit is belangrijk, omdat het wenselijk is in een vergelijkende rassenproef dezelfde weersomstandigheden te hebben in hetzelfde ontwikkelingsstadium, bijvoorbeeld tijdens de bloei, zaadvulling of oogst. Elk ras is afkomstig van een ander kweekbedrijf, en de rassen variëren in ouderdom.

Gezaaide velden

De rassen verschilden betrouwbaar en reproduceerbaar voor zaadopbrengst (hoofdstuk 5). Ze vertoonden ook reproduceerbare verschillen voor duizend-korrel gewicht, maar de rangorde van de rassen voor zaadopbrengst en duizend-korrel gewicht verschilde. Er waren geen rasverschillen voor kiemkracht van het geoogste zaad. De verschillen voor zaadopbrengst konden niet worden verklaard uit bovengrondse droge stofproduktie (hoofdstuk 6). Er waren geen betrouwbare rasverschillen voor verdeling van droge stof over blad, stengel en aar. De berekende kenmerken 'aantal zaden per oppervlakte-eenheid' en 'oogstindex' (schoon zaad gedeeld door totale biomassa op basis van droge stof) vertoonden de beste correlatie met zaadopbrengst.

In velden zijn geen bruikbare kenmerken voor indirekte selektie gevonden die gecorreleerd waren met zaadopbrengst. Kwekers kunnen dus niet de zaadopbrengst voorspellen op basis van een ander kenmerk, bepaald in kleine veldjes: de zaadopbrengst zèlf moet worden bepaald in gezaaide velden.

Aparte planten

Een andere mogelijkheid zou zijn, aparte planten te selekteren. Dit zou in een vroegtijdig stadium van de selektie kunnen, omdat kwekers hun veredelingsprogramma veelal met aparte planten beginnen. Indirekte selektie in aparte planten is mogelijk als kenmerken worden opgespoord, die een hoge erfelijkheidsgraad hebben en een hoge correlatie met de zaadopbrengst in een gezaaid veld.

Binnen elk ras werd grote genotypische variatie gevonden voor zaadopbrengst en -opbrengstcomponenten en voor andere kenmerken tussen de aparte planten (hoofdstuk 8). Zaadopbrengst per plant was gecorreleerd met vroegheid, halmaantal en zaadopbrengst per halm in aparte planten. Bij selektie van planten met een late

schietdatum moet extra aandacht worden geschonken aan de zaadopbrengst.
Bij selektie in kruisbevruchters gaat het echter niet primair om de individuele prestaties, maar er moeten ouders geselekteerd worden die superieure nakomelingen produceren. De erfelijkheidsgraad van zaadopbrengst en -opbrengstcomponenten is in drie rassen bestudeerd aan de 50 oudergenotypen in duplo en hun nakomelingen (half-sib families) à 2 individuen (hoofdstuk 7). De erfelijkheidsgraad in brede zin was in het algemeen hoog. De erfelijkheidsgraad in engere zin is zowel berekend op basis van de variantiecomponenten tussen families als op ouder-nakomelingschaps regressies. De eerste methode leverde voor zeer weinig kenmerken betrouwbare variatie op, slechts voor vlagbladbreedte en bloeidatum was de erfelijkheidsgraad hoog. De tweede methode leverde ook betrouwbare schattingen op voor schietdatum, aantal bloempakjes en aarlengte. Deze kenmerken bieden daarom in aparte planten perspektieven voor selektiemethoden zoals massaselektie, waarbij vooral additieve genetische variatie wordt benut. Om als indirekt selektiekenmerk voor zaadopbrengst in een veld bruikbaar te zijn, moet er behalve een hoge erfelijkheidsgraad tevens een goede correlatie zijn met zaadopbrengst in een veld.
Rassen met een vergelijkbare zaadproduktie in een veld, bleken echter als aparte planten heel verschillend te reageren (hoofdstuk 8). Goede of slechte rassen konden daarom niet goed worden gekarakteriseerd op basis van aparte planten. Aparte planten van goede rassen hadden in het algemeen een kleinere plantomvang tijdens doorschieten en een iets hogere gemiddelde zaadopbrengst per halm dan aparte planten van slechte rassen. Hoewel de rassen verschilden op grond van hun gemiddelde over 25 of 50 planten, was de variatie binnen elk ras veel groter dan de variatie tussen rassen. In aparte planten zijn daarom geen bruikbare kenmerken gevonden voor indirekte selektie voor zaadopbrengst in een gezaaid veld.

Perspektieven voor veredeling
Er zijn betrouwbare rasverschillen aangetoond voor zaadproduktie in gezaaide velden. Deze verschillen waren reproduceerbaar over diverse milieus. Het was echter niet mogelijk rassen met een contrasterende zaadopbrengst te onderscheiden op grond van hun groeiritme, droge stofproduktie, verdeling van droge stof, halmaantal of andere gewasfysiologiche kenmerken. Op veldniveau konden geen onafhankelijk bepaalde kenmerken worden aangetoond die correleerden met zaadopbrengst.
Binnen elk van de rassen was grote variatie tussen afzonderlijke planten.
Op basis van aparte planten was de erfelijkheidsgraad van de meeste kenmerken laag, maar vroegheid, aarlengte, aantal bloempakjes per halm en vlagbladbreedte hadden een hoge erfelijkheidsgraad.
Indirekte selektie op basis van aparte planten met als doel verhoging van de zaadopbrengst in een veld is waarschijnlijk weinig zinvol, omdat de kenmerken die een hoge erfelijkheidsgraad hadden, gemiddeld op rasbasis geen verband vertoonden met zaadopbrengst in een veld. De erfelijkheidsgraad is echter onderzocht op basis van aparte planten en planten zijn veel variabeler dan velden. Daarom wordt de relatie tussen de zaadopbrengst van nakomelingen in een gezaaid veld en eigenschappen van hun moeders als aparte plant momenteel verder onderzocht.

CURRICULUM VITAE

Anjo Elgersma werd op 27 juli 1959 geboren te Schraard (Fr.). Zij behaalde het Atheneum-B diploma in 1977 aan het Jan-Brugmancollege te Bolsward. In 1985 werd het Ingenieursdiploma aan de toenmalige Landbouwhogeschool te Wageningen behaald in de richting Plantenveredeling met als verdere specialisaties Landbouwplantenteelt & Graslandkunde en Erfelijkheidsleer. Van juni 1984 t/m december 1988 was de auteur in tijdelijke dienst van het Hoofdproduktschap voor Akkerbouwprodukten en was gedetacheerd bij de Stichting Voor Plantenveredeling (SVP) in Wageningen, waar zij veredelingsonderzoek verrichtte aan zaadopbrengst in grassen. Dit onderzoek heeft ondermeer geleid tot het schrijven van dit proefschrift. Sinds 1989 is de auteur in dienst van de Stichting Nederlands Graan-Centrum te Wageningen en verricht op de SVP (sinds 1 januari 1990 opgegaan in het Centrum voor Plantenveredelingsonderzoek CPO) onderzoek aan stresstolerantie bij de zaadproduktie van Engels raaigras.